

TRANSMITTAL LETTER TO THE UNITED STATES
DESIGNATED/ELECTED OFFICE (DO/EO/US)
CONCERNING A FILING UNDER 35 U.S.C. 371ATTORNEY'S DOCKET NUMBER
WALLACH=21

U.S. APPLICATION NO. (If known, see 37 CFR 1.5

09/155676

INTERNATIONAL APPLICATION NO.
PCT/IL97/00117INTERNATIONAL FILING DATE
01 April 1997PRIORITY DATE (If known)
02 April 1996

TITLE OF INVENTION

MODULATORS OF TNF RECEPTOR ASSOCIATED FACTOR (TRAF), THEIR PREPARATION AND USE

APPLICANT(S) FOR DO/EO/US
David WALLACH et al.

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a FIRST submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a SECOND or SUBSEQUENT submission of items concerning a filing under 35 U.S.C. 371.
3. ☒ This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. ☒ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. ☒ A copy of the International Application as filed (35 U.S.C. 371(c)(2))
- a. ☐ is transmitted herewith (required only if not transmitted by the International Bureau).
- b. ☒ has been transmitted by the International Bureau.
- c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☐ A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
- a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
- b. ☐ have been transmitted by the International Bureau.
- c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
- d. ☒ have not been made and will not be made.
8. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☐ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. ☐ A translation of the annexes of the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 16. below concern document(s) or information included:

11. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. ☒ A FIRST preliminary amendment.
- ☒ A SECOND or SUBSEQUENT preliminary amendment.
14. ☐ A substitute specification.
15. ☐ A change of power of attorney and/or address letter.
16. ☒ Other items or information:
1. A courtesy copy of the specification as originally filed.
 2. A courtesy copy of the first page of the International Publication (WO97/37016).
 3. A courtesy copy of the International Search Report.
 4. A courtesy copy of the International Preliminary Examination Report.
 5. Formal drawings, 76 sheets, figures 1-7.

PCT/IL97/00117

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17. ☒ The following fees are submitted:

BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)):

Neither international preliminary examination fee (37 CFR 1.482)
nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO
and International Search Report not prepared by the EPO or JPO \$1070.00International preliminary examination fee (37 CFR 1.482) not paid to
USPTO but International Search Report prepared by the EPO or JPO \$930.00International preliminary examination fee (37 CFR 1.482) not paid to USPTO
but international search fee (37 CFR 1.445(a)(2)) paid to USPTO \$790.00International preliminary examination fee (37 CFR 1.482) paid to USPTO
but all claims did not satisfy provisions of PCT Article 33(1)-(4) \$720.00International preliminary examination fee (37 CFR 1.482) paid to USPTO
and all claims satisfied provisions of PCT Article 33(1)-(4) \$98.00

ENTER APPROPRIATE BASIC FEE AMOUNT =

CALCULATIONS PTO USE ONLY

\$ 930.00

Surcharge of \$130.00 for furnishing the oath or declaration later than ☐ 20 ☒ 30
months from the earliest claimed priority date (37 CFR 1.492(e)).

\$

CLAIMS NUMBER FILED NUMBER EXTRA RATE

Total claims 50 - 20 = 30 x \$22.00 \$

Independent claims 6 - 3 = 3 x \$82.00 \$

MULTIPLE DEPENDENT CLAIM(S) (if applicable) + \$270.00 \$

TOTAL OF ABOVE CALCULATIONS =

\$ 930.00

Reduction of 1/2 for filing by small entity, if applicable. A Small Entity Statement
must also be filed (Note 37 CFR 1.9, 1.27, 1.28). +

\$

SUBTOTAL =

\$ 930.00

Processing fee of \$130.00 for furnishing the English translation later than ☐ 20 ☐ 30
months from the earliest claimed priority date (37 CFR 1.492(f)).

\$

TOTAL NATIONAL FEE =

\$ 930.00

Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be
accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). \$40.00 per property +

\$

TOTAL FEES ENCLOSED =

\$ 930.00

Amount to be
refunded: \$

charged: \$

a. ☒ A check in the amount of \$ 930.00 to cover the above fees is enclosed.b. ☐ Please charge my Deposit Account No. _____ in the amount of \$ _____ to cover the above fees.
A duplicate copy of this sheet is enclosed.c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any
overpayment to Deposit Account No. 02-4035. A duplicate copy of this sheet is enclosed.NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR
1.137 (a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

BROWDY AND NEIMARK, P.L.L.C.
419 Seventh Street N.W., Suite 300
Washington, D.C. 20004

SIGNATURE

Roger L. Browdy

NAME

25,618

REGISTRATION NUMBER

Date of this submission: October 2, 1998

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:)	Art Unit:
David WALLACH et al.)	
IA No.: PCT/IL97/00117)	
IA Filed: 01 April 1997)	Washington, D.C.
U.S. App. No.:)	
(Not Yet Assigned))	
National Filing Date:)	October 2, 1998
(Not Yet Received))	
For: MODULATORS OF TNF...)	Docket No.: WALLACH=21

PRELIMINARY AMENDMENT

Honorable Commissioner of Patents and Trademarks
Washington, D.C. 20231

Sir:

Contemporaneous with the filing of this case and prior to calculation of the filing fee, kindly amend as follows:

IN THE CLAIMS

Claim 4, line 1, delete "any one of claims 1 to 3", and insert therefor --claim 1--.

Claim 5, line 1, delete "any one of claims 1 to 4", and insert therefor --claim 1--.

Claim 6, line 1, delete "any one of claims 1 to 4", and insert therefor --claim 1--.

Claim 8, line 1, delete "or 6".

Claim 11, line 1, delete "or 10".

Claim 13, line 1, delete "any one of claims 1-12", and insert therefor --claim 1--.

Claim 16, line 2, delete "any one of claims 13-15", and insert therefor --claim 13--.

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Claim 17, line 2, delete "any one of claims 1-12", and insert therefor --claim 1--.

Claim 23, line 4, delete "any one of claims 17-20", and insert therefor --claim 17--.

Claim 27, line 4, delete "any one of claims 1-11", and insert therefor --claim 1--.

Claim 29, line 4, delete "any one of claims 17-20", and insert therefor --claim 17--.

Claim 30, lines 1 and 2, delete "any one of claims 17-20", and insert therefor --claim 17--.

Claim 31, line 1, delete "any one of claims 23-30", and insert therefor --claim 23--.

Claim 32, line 3, delete "any one of claims 17-20", and insert therefor --claim 17--.

Claim 33, lines 4 and 5, delete "any one of claims 17-20", and insert therefor --claim 17--.

Claim 34, lines 3 and 4, delete "any one of claims 1-11", and insert therefor --claim 1--.

Claim 35, line 3, delete "any one of claims 17-20", and insert therefor --claim 17--.

Claim 36, line 3, delete "any one of claims 17-20", and insert therefor --claim 17--.

Claim 39, line 4, delete "or 20".

Claim 40, line 3, delete "any one of claims 17-20", and insert therefor --claim 17--;

lines 5 and 6, delete "any one of claims 17-20", and insert therefor --claim 17--;

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line 8, delete "any one of claims 17-20", and
insert therefor --claim 17--; and

line 10, delete "any one of claims 17-20", and
insert therefor --claim 17--.

Claim 43, line 2, delete "any one of claims 17-20", and
insert therefor --claim 17--.

Claim 44, line 2, delete "any one of claims 17-20", and
insert therefor --claim 17--.

Claim 46, line 2, delete "any one of claims 17-20", and
insert therefor --claim 17--.

Claim 49, lines 2 and 3, delete "any one of claims 17-
20", and insert therefor --claim 17--; and

line 5, delete "any one of claims 17-20", and
insert therefor --claim 17--.

REMARKS

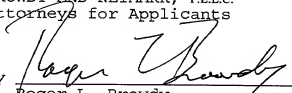
The above amendments to the claims are being made in
order to eliminate any properly multiply dependent claims, for the
purpose of reducing the filing fee. Please enter this amendment
prior to calculation of the filing fee in this case.

Favorable consideration and allowance are earnestly
solicited.

Respectfully submitted,

BROWDY AND NEIMARK, P.L.L.C.
Attorneys for Applicants

By


Roger L. Browdy
Registration No. 25,618

09/155676
10 Rec'd PCT/PTO 02 OCT 1998

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:)	Art Unit:
David WALLACH et al.)	
IA No.: PCT/IL97/00117)	
IA Filed: 01 April 1997)	Washington, D.C.
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(Not Yet Received))	
For: MODULATORS OF TNF...)	Docket No.: WALLACH=21

SUPPLEMENTAL PRELIMINARY AMENDMENT

Honorable Commissioner of Patents and Trademarks
Washington, D.C. 20231

Sir:

Prior to examination on the merits, kindly amend as follows:

IN THE CLAIMS

Please amend the claims as follows:

19 (amended). A TRAF-binding protein, isoforms, fragments, analogs and derivatives thereof [, according to claim 17 being the] encoded by a DNA sequence according to claim 1, said protein, isoforms, fragments, analogs and derivatives thereof being capable of binding to at least the portion or the TRAF2 protein between amino acids 222-501 of TRAF2, wherein said protein is NIK protein, isoforms, analogs, fragments [and] or derivatives thereof [encoded by the DNA sequence according to any one of claims 1-12].

Claim 21, line 1, after "producing a", insert -- TRAF-binding --; and

line 2, delete "according to any one of claims 17-19".

Claim 22, lines 2-4, delete "or 18;" to the end of the claim.

Claim 25, line 7 and 8, delete "according to any one of claims 17-20".

28 (amended). A method according to claim 27 wherein said oligonucleotide sequence is introduced to said cells via a recombinant animal virus vector carrying a sequence encoding a viral surface protein (ligand) that is capable of binding to a specific cell surface receptor on the surface of said cells to be treated and a [of claim 25 wherein said] second sequence [of said virus encodes] encoding said oligonucleotide sequence.

Please add the following new claim:

50. Antibodies or active fragments or derivatives thereof, specific for the NIK protein, isoform, analog, fragment or derivative thereof according to claim 19.

REMARKS

Claims 1-50 presently appear in this case. The present amendments are being made in order to correct the improper

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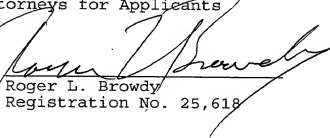
multiply dependent claims and to place them in singly dependent form.

Favorable consideration and allowance are earnestly solicited.

Respectfully submitted,

BROWDY AND NEIMARK, P.L.L.C.
Attorneys for Applicants

By


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300 Rec'd PCT/PTO 02 OCT 1998

**MODULATORS OF TNF RECEPTOR ASSOCIATED FACTOR
(TRAF), THEIR PREPARATION AND USE**

5 **Field of the Invention**

The present invention concerns DNA sequences encoding proteins capable of binding to TRAF2, the proteins encoded thereby, and the use of said proteins and DNA sequences in the treatment or prevention of a pathological condition associated with NF- κ B induction or with any other activity mediated by TRAF2 or by other molecules to which
10 said proteins bind.

Background of the Invention

The Tumor Necrosis Factor/Nerve Growth Factor (TNF/NGF) receptor superfamily is defined by structural homology between the extracellular domains of its
15 members (Bazan, 1993; Beutler and van Huffel, 1994; Smith et al., 1994). Except for two receptors, the p55 TNF receptor and Fas/APO1, the various members of this receptor family do not exhibit clear similarity of structure in their intracellular domains. Nevertheless, there is much similarity of function between the receptors, indicating that they share common signaling pathways. One example for this similarity is the ability of
20 several receptors of the TNF/NGF family to activate the transcription factor NF- κ B. This common ability was ascribed to a capability of a cytoplasmic protein that activates NF- κ B, TNF Receptor Associated Factor 2 (TRAF2) to bind to the structurally-dissimilar intracellular domains of several of the receptors of the TNF/NGF family. By what mechanisms does TRAF2 act and how is its responsiveness to the different receptors to
25 which it binds coordinated is not known.

TRAF2 is a member of a recently described family of proteins called TRAF that includes several proteins identified as, for example, TRAF1, TRAF2 (Rothe, M., Wong, s.c., Henzel, W.J. and Goeddel, D (1994) Cell 78:681-692; PCT published application WO 95/33051), TRAF3 (Cheng, G. et al. (1995)), and TRAF6 (see Cao et al., 1996a).

30 All proteins belonging to the TRAF family share high degree of amino acid identity in their C-terminal domains, while their N-terminal domains may be unrelated. As shown in a schematic illustration of TRAF2 (Fig. 1), the molecule contains a ring finger motif and two TFIIIA-like zinc finger motifs at its C-terminal area. The C-terminal half of the molecule

includes a region known as the "TRAF domain" containing a potential leucine zipper region extending between amino acids 264 - 358 (called N-TRAF), and another part towards the carboxy end of the domain between amino acids 359 - 501 (called C-TRAF) which is responsible for TRAF binding to the receptors and to other TRAF molecules to form homo- or heterodimers.

Activation of the transcription factor NF- κ B is one manifestation of the signaling cascade initiated by some of the TNF/NGF receptors and mediated by TRAF2. NF- κ B comprises members of a family of dimer-forming proteins with homology to the Rel oncogene which, in their dimeric form, act as transcription factors. These factors are ubiquitous and participate in regulation of the expression of multiple genes. Although initially identified as a factor that is constitutively present in B cells at the stage of Ig κ light chain expression, NF- κ B is known primarily for its action as an inducible transcriptional activator. In most known cases NF- κ B behaves as a primary factor, namely the induction of its activity is by activation of pre-existing molecules present in the cell in their inactive form, rather than its de-novo synthesis which in turn relies on inducible transcription factors that turn-on the NF- κ B gene. The effects of NF- κ B are highly pleiotropic. Most of these numerous effects share the common features of being quickly induced in response to an extracellular stimulus. The majority of the NF- κ B-activating agents are inducers of immune defense, including components of viruses and bacteria, cytokines that regulate immune response, UV light and others. Accordingly, many of the genes regulated by NF- κ B contribute to immune defense (see Blank et al., 1992; Grilli et al., 1993; Baeuerle and Henkel, 1994, for reviews).

One major feature of NF- κ B-regulation is that this factor can exist in a cytoplasmic non-DNA binding form which can be induced to translocate to the nucleus, bind DNA and activate transcription. This dual form of the NF- κ B proteins is regulated by I- κ B - a family of proteins that contain repeats of a domain that has initially been-discerned in the erythrocyte protein ankyrin (Gilmore and Morin, 1993). In the unstimulated form, the NF- κ B dimer occurs in association with an I- κ B molecule which imposes on it cytoplasmic location and prevents its interaction with the NF- κ B-binding DNA sequence and activation of transcription. The dissociation of I- κ B from the NF- κ B dimer constitutes the critical step of its activation by many of its inducing agents (DiDonato et al., 1995). Knowledge of the

mechanisms that are involved in this regulation is still limited. There is also just little understanding of the way in which cell specificity in terms of responsiveness to the various NF- κ B-inducing agents is determined.

One of the most potent inducing agents of NF- κ B is the cytokine tumor necrosis factor (TNF). There are two different TNF receptors, the p55 and p75 receptors. Their expression levels vary independently among different cells (Vandenebeele et al., 1995). The p75 receptor responds preferentially to the cell-bound form of TNF (TNF is expressed both as a beta-transmembrane protein and as a soluble protein) while the p55 receptor responds just as effectively to soluble TNF molecules (Grell et al., 1995). The intracellular domains of the two receptors are structurally unrelated and bind different cytoplasmic proteins. Nevertheless, at least part of the effects of TNF, including the cytotoxic effect of TNF and the induction of NF- κ B, can be induced by both receptors. This feature is cell specific. The p55 receptor is capable of inducing a cytotoxic effect or activation of NF- κ B in all cells that exhibit such effects in response to TNF. The p75-R can have such effects only in some cells. Others, although expressing the p75-R at high levels, show induction of the effects only in response to stimulation of the p55-R (Vandenebeele et al., 1995). Apart from the TNF receptors, various other receptors of the TNF/NGF receptor family: CD30 (McDonald et al., 1995), CD40 (Berberich et al., 1994; Lalmannach-Girard et al., 1993), the lymphotoxin beta receptor and, in a few types of cells, Fas/APO1 (Rensing-Ehl et al., 1995), are also capable of inducing activation of NF- κ B. The IL-1 type I receptor, also effectively triggering NF- κ B activation, shares most of the effects of the TNF receptors despite the fact that it has no structural similarity to them.

The activation of NF- κ B upon triggering of these various receptors results from induced phosphorylation of its associated I- κ B molecules. This phosphorylation tags I- κ B to degradation, which most likely occurs in the proteasome. The nature of the kinase that phosphorylates I- κ B, and its mechanism of activation upon receptor triggering is still unknown. However, in the recent two years some knowledge has been gained as to the identity of three receptor-associated proteins that appear to take part in initiation of the phosphorylation (see diagrammatic illustration in Figures 2a and 6). A protein called TRAF2, initially cloned by D. Goeddel and his colleagues (Rothe et al., 1994), seems to play a central role in NF- κ B-activation by the various receptors of the TNF/NGF family. The protein, which when expressed at high levels can by itself trigger NF- κ B activation,

binds to activated p75 TNF-R (Rothe et al., 1994), lymphotoxin beta receptor (Mosialos et al., 1995), CD40 (Rothe et al., 1995a) and CD-30 (unpublished data) and mediates the induction of NF- κ B by them. TRAF2 does not bind to the p55 TNF receptor nor to Fas/APO1, however, it can bind to a p55 receptor-associated protein called TRADD and TRADD has the ability to bind to a Fas/APO1-associated protein called MORT1 (or FADD - see Boldin et al. 1995b and 1996). Another receptor-interacting protein, called RIP (see Stanger et al., 1995) is also capable of interacting with TRAF2 as well as with FAS/APO1, TRADD, the p55 TNF receptor and MORT-1. Thus, while RIP has been associated with cell cytotoxicity induction (cell death), its ability to interact with TRAF2 also implicates it in NF- κ B activation and it also may serve in addition to augment the interaction between FAS/APO1, MORT-1, p55 TNF receptor and TRADD with TRAF2 in the pathway leading to NF- κ B activation. These associations apparently allow the p55 TNF receptor and Fas/APO1 to trigger NF- κ B activation (Hsu et al., 1995; Boldin et al., 1995; Chinnalyan et al., 1995; Varfolomeev et al., 1996; Hsu et al., 1996). The triggering of NF- κ B activation by the IL-1 receptor occurs independently of TRAF2 and may involve a recently-cloned IL-1 receptor-associated protein-kinase called IRAK (Croston et al., 1995).

By what mechanism TRAF2 acts is not clear. Several cytoplasmic molecules that bind to TRAF2 have been identified (Rothe et al., 1994; Rothe et al., 1995b). However, the information on these molecules does not provide any clue as to the way by which TRAF2, which by itself does not possess any enzymatic activity, triggers the phosphorylation of I- κ B. There is also no information yet of mechanisms that dictate cell-specific pattern of activation of TRAF2 by different receptors, such as observed for the induction of NF- κ B by the two TNF receptors.

In addition to the above mentioned, of the various TRAF proteins, it should also be noted that TRAF2 binds to the p55 (CD120a) and p75 (CD120b) TNF receptors, as well as to several other receptors of the TNF/NGF receptor family, either directly or indirectly via other adaptor proteins as noted above, for example with reference to the FAS/APO1 receptor, and the adaptor proteins MORT-1, TRADD and RIP. As such, TRAF2 is crucial for the activation of NF- κ B (see also Wallach, 1996). However, TRAF3 actually inhibits activation of NF- κ B by some receptors of the TNF/NGF family (see Rothe et al., 1995a), whilst TRAF6 is required for induction of NF- κ B by IL-1 (see Cao et al., 1996a).

Accordingly, as regards NF- κ B activation and its importance in maintaining cell viability, the various intracellular pathways involved in this activation have heretofore not been clearly elucidated, for example, how the various TRAF proteins, are involved directly or indirectly.

Furthermore, as is now known regarding various members of the TNF/NGF receptor family and their associated intracellular signaling pathways inclusive of various adaptor, mediator/modulator proteins (see brief reviews and references in, for example, co-pending co-owned Israel Patent Application Nos. 114615, 114986, 115319, 116588), TNF and the FAS/APO1 ligand, for example, can have both beneficial and deleterious effects on cells. TNF, for example, contributes to the defence of the organism against tumors and infectious agents and contributes to recovery from injury by inducing the killing of tumor cells and virus-infected cells, augmenting antibacterial activities of granulocytes, and thus in these cases the TNF-induced cell killing is desirable. However, excess TNF can be deleterious and as such TNF is known to play a major pathogenic role in a number of diseases such as septic shock, anorexia, rheumatic diseases, inflammation and graft-vs-host reactions. In such cases TNF-induced cell killing is not desirable. The FAS/APO1 ligand, for example, also has desirable and deleterious effects. This FAS/APO1 ligand induces via its receptor the killing of autoreactive T cells during maturation of T cells, i.e. the killing of T cells which recognize self-antigens, during their development and thereby preventing autoimmune diseases. Further, various malignant cells and HIV-infected cells carry the FAS/APO1 receptor on their surface and can thus be destroyed by activation of this receptor by its ligand or by antibodies specific thereto, and thereby activation of cell death (apoptosis) intracellular pathways mediated by this receptor. However, the FAS/APO1 receptor may mediate deleterious effects, for example, uncontrolled killing of tissue which is observed in certain diseases such as acute hepatitis that is accompanied by the destruction of liver cells.

In view of the above, namely, that receptors of the TNF/NGF family can induce cell death pathways on the one hand and can induce cell survival pathways (via NF- κ B induction) on the other hand, there apparently exists a fine balance, intracellularly between these two opposing pathways. For example, when it is desired to achieve maximal destruction of cancer cells or other infected or diseased cells, it would be desired to have TNF and/or the FAS/APO1 ligand inducing only the cell death pathway without inducing

NF- κ B. Conversely, when it is desired to protect cells such as in, for example, inflammation, graft-vs-host reactions, acute hepatitis, it would be desirable to block the cell killing induction of TNF and/or FAS/APO1 ligand and enhance, instead, their induction of NF- κ B. Likewise, in certain pathological circumstances it would be desirable to block the intracellular signaling pathways mediated by the p75 TNF receptor and the IL-1 receptor, while in others it would be desirable to enhance these intracellular pathways.

Summary of the Invention

It is an object of the invention to provide novel proteins, including all isoforms, analogs, fragments or derivatives thereof which are capable of binding to the tumor necrosis factor receptor-associated (TRAF) proteins. As the TRAF proteins are involved in the modulation or mediation of the activation of the transcription factor NF- κ B, which is initiated by some of the TNF/NGF receptors, as well as others as noted above, the novel proteins of the present invention by binding to TRAF proteins are therefore capable of affecting (modulating or mediating) the intracellular signaling processes initiated by various ligands (e.g. TNF, FAS ligand and others) binding to their receptors such as, for example, their modulation/mediation of NF- κ B activation, via interaction directly or indirectly with TRAF proteins.

The novel proteins of the present invention are therefore direct modulators/mediators of the intracellular biological activity of TRAF proteins (e.g. induction of NF- κ B activation by TRAF2 and TRAF6 and inhibition of NF- κ B activation, by TRAF3).

The novel proteins of the invention are likewise indirect modulators/mediators of the intracellular biological activity of a variety of other proteins which are capable of interacting with TRAF proteins directly or indirectly (e.g. FAS/APO1 receptor, p55 TNF receptor, p75 TNF receptor, IL-1 receptor and their associated proteins, such as, for example, MORT-1, TRADD, RIP).

Another object of the invention is to provide antagonists (e.g. antibodies, peptides, organic compounds, or even some isoforms) to the above novel TRAF-binding proteins, including isoforms, analogs, fragments and derivatives thereof, which may be used to inhibit the signaling process, or, more specifically, to inhibit the activation of NF- κ B and its associated involvement in cell-survival processes, when desired. Likewise, when the TRAF-

binding proteins of the invention or the TRAF protein to which they bind (e.g. TRAF3) are themselves inhibitory for NF- κ B activation, then it is an object to provide antagonists to these TRAF-binding proteins to activate the signaling process or more specifically, to block the inhibition of NF- κ B activation and hence bring about enhanced NF- κ B activation, when desired.

A further object of the invention is to use the above novel TRAF-binding proteins, isoforms, analogs, fragments and derivatives thereof, to isolate and characterize additional proteins or factors, which may be involved in regulation of TRAF protein activity and/or the above noted receptor activity, e.g. other proteins which may bind to TRAF proteins and influence their activity, and/or to isolate and identify other receptors or other cellular proteins further upstream or downstream in the signaling process(es) to which these novel proteins, analogs, fragments and derivatives bind, and hence, in whose function they are also involved.

A still further object of the invention is to provide inhibitors which can be introduced into cells to bind or interact with the novel TRAF-binding proteins and possible isoforms thereof, which inhibitors may act to inhibit TRAF protein-associated activity in, for example, NF- κ B activation and hence, when desired, to inhibit NF- κ B activation; or which may act to inhibit inhibitory TRAF-associated activity (e.g. TRAF3) in NF- κ B activation and hence, when desired, to enhance NF- κ B activation.

Moreover, it is an object of the present invention to use the above-mentioned novel TRAF-binding proteins, isoforms and analogs, fragments and derivatives thereof as antigens for the preparation of polyclonal and/or monoclonal antibodies thereto. The antibodies, in turn, may be used, for example, for the purification of the new proteins from different sources, such as cell extracts or transformed cell lines.

Furthermore, these antibodies may be used for diagnostic purposes, e.g. for identifying disorders related to abnormal functioning of cellular effects mediated directly by TRAF proteins or mediated by the p55 TNF receptor, FAS/APO1 receptor, or other related receptors and their associated cellular proteins (e.g. MORT-1, TRADD, RIP), which act directly or indirectly to modulate/mediate intracellular processes via interaction with TRAF proteins.

A further object of the invention is to provide pharmaceutical compositions comprising the above novel TRAF proteins, isoforms, or analogs, fragments or derivatives

thereof, as well as pharmaceutical compositions comprising the above noted antibodies or other antagonists.

In accordance with the present invention, a number of novel TRAF-binding proteins, in particular, TRAF2-binding proteins, have been isolated. These TRAF2-binding proteins have high specificity of binding to TRAF2 (see Examples below) and hence are modulators or mediators of TRAF2 intracellular activity. TRAF2 is involved in the modulation or mediation of at least one intracellular signaling pathway being the cell survival- or viability- related pathway in which Traf 2 is directly involved in activation of NF- κ B which plays a central role in cell survival. In fact, one of these new proteins, called NIK (for 'NF- κ B inducing kinase') binds to TRAF2 and stimulates NF- κ B activity. NIK is a kinase sharing sequence similarity with several MAPKK kinases (see below). Further, TRAF2 by being capable of interaction directly or indirectly with the above noted p55 TNF receptor, p75 TNF receptor, FAS/APO1 receptors and their associated proteins MORT-1, TRADD and RIP, also is a mediator or modulator of the NF- κ B induction or activation activity attributed to these receptors. TRAF2 is therefore a modulator/mediator of the cell survival pathways (as opposed to the cell death pathways) mediated by these receptors and their associated proteins and as such the extent of interaction between these receptors and/or proteins with TRAF2 is an important factor in the outcome of the activity of these receptors (once activated by their ligands), namely, whether the cells will survive or die. Accordingly, the proteins of the invention, for example, NIK, play a key role in this interaction between TRAF2 and the other proteins/receptors with which TRAF2 interacts, as proteins such as NIK by binding specifically to TRAF2 will modulate its activity and/or will have their activity modulated by interaction with TRAF2.

The TRAF-binding proteins, such as, for example, the TRAF2-binding proteins, including NIK, have been isolated and cloned using the two-hybrid system, partially and fully sequenced, and characterized, and as is detailed herein below appear to be highly specific TRAF2-binding proteins, and hence specific TRAF2 modulators/mediators.

As will be used herein throughout, TRAF protein activity, for example TRAF2 activity, is meant to include its activity in modulation/mediation in the cell survival pathway, especially as concerns NF- κ B induction/activation. Likewise, as used herein throughout TRAF-binding protein, in particular TRAF2-binding protein, activity is meant to include their modulation/mediation of TRAF-, in particular, TRAF2- activity by virtue of

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their specific binding to TRAF, especially TRAF2 proteins, this modulation/mediation including modulation/mediation of cell survival pathways, in particular, those relating to NF- κ B activation/induction in which TRAF proteins, especially TRAF2 is involved directly or indirectly and as such TRAF or TRAF2-binding protein may be considered as indirect modulator/mediators of all the above mentioned proteins and possibly a number of others which are involved in cell survival, especially NF- κ B activation/induction and to which TRAF2 (or other TRAF proteins) binds, or with which TRAF2 (or other TRAF proteins) interacts in a direct or indirect fashion.

Accordingly, the present invention provides a DNA sequence encoding a protein capable of binding to a tumor necrosis factor receptor-associated (TRAF) molecule.

One embodiment of the DNA sequence of the invention is a sequence encoding a protein capable of binding to TRAF2.

Another embodiment of the DNA sequence of the invention is a sequence encoding a protein capable of binding to at least the amino acid residues 222-501 of the amino acid sequence of TRAF2

Other embodiments of the DNA sequence of the invention include :

(a) a cDNA sequence of the herein designated clone 9 comprising the nucleotide sequence depicted in Fig 3a.;

(b) a cDNA sequence of the herein designated clone 10 comprising the nucleotide sequence depicted in Fig 4;

(c) a cDNA sequence of the herein designated clone 15 comprising the nucleotide sequence depicted in Fig. 5a;

(d) a fragment of a sequence (a)-(c) which encodes a biologically active protein capable of binding to least the 222-501 amino acid sequence of TRAF2;

(e) a DNA sequence capable of hybridization to a sequence of (a)-(d) under moderately stringent conditions and which encodes a biologically active protein capable of binding to at least the 222-501 amino acid sequence of TRAF2; and

(f) a DNA sequence which is degenerate as a result of the genetic code to the DNA sequences defined in (a)-(e) and which encodes a biologically active protein capable of binding to at least the 222-501 amino acid sequence of TRAF2.

Yet other embodiments of the DNA sequence of the invention noted above include :

10

A DNA sequence selected from the sequences contained in the herein designated cDNA clones 9 and 15;

A DNA sequence which encodes a protein that also modulates NF- κ B activity; and

5 A DNA sequence selected from the sequences contained in the herein designated cDNA clone 10.

An additional preferred embodiment of the above DNA sequences of the invention is a DNA sequence comprising the DNA sequence encoding the protein NIK (for 'NF- κ B inducing kinase').

10 Embodiments of the above DNA sequence of the invention encoding the protein NIK include :

(i) A DNA sequence encoding the protein NIK, isoforms, fragments or analogs thereof, said NIK, isoforms, fragments or analogs thereof being capable of binding to TRAF2 and which is capable of modulating the activity of NF- κ B;

15 (ii) A DNA sequence as in (i) above, selected from the group consisting of :

a) a cDNA sequence derived from the coding region of a native NIK protein;

b) DNA sequences capable of hybridization to a sequence of (a) under moderately stringent conditions and which encode a biologically active NIK; and

20 c) DNA sequences which are degenerate as a result of the genetic code to the sequences defined in (a) and (b) and which encode a biologically active NIK protein;

(iii) A DNA sequence as in (i) or (ii) above comprising at least part of the sequence depicted in Fig. 6 and encoding at least one active NIK protein, isoform, analog or fragment;

25 (iv) A DNA sequence as in (iii) above encoding a NIK protein, isoform, analog or fragment having at least part of the amino acid sequence depicted in Fig. 6.

In another aspect, the invention provides proteins or polypeptides encoded by the above noted DNA coding sequences of the invention, the isoforms, analogs, fragments and derivatives of said proteins and polypeptides, provided that they are capable of binding to TRAF2, preferably to at least the 222-501 amino acid sequenced of TRAF2. Embodiments of these proteins/polypeptides, and their isoforms, analogs, fragments and derivatives according to the invention include :

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(a) a protein being the protein encoded by herein designated clone ..

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(b) a protein, isoforms, fragments, analogs and derivatives thereof, being the NIK protein, isoforms, analogs, fragments and derivatives thereof encoded by the above noted DNA sequences encoding said NIK protein, isoforms, analogs, fragments and derivatives; and

(c) a NIK protein, isoforms, analogs, fragments and derivatives thereof being the NIK protein, isoforms, analogs, fragments and derivatives thereof encoded by the above noted DNA sequences encoding said NIK protein, isoforms, analogs, fragments and derivatives, wherein said protein, isoforms, fragments and derivatives have at least part of the amino acid sequence depicted in Fig. 6.

In yet another aspect, the invention provides a vector comprising any of the above DNA sequences according to the invention which are capable of being expressed in host cells selected from prokaryotic and eukaryotic cells; and the transformed prokaryotic and eukaryotic cells containing said vector.

The invention also provides a method for producing a protein, isoform, analog, fragment or derivative encoded by any of the above DNA sequences according to the invention which comprises growing the above mentioned transformed host cells under conditions suitable for the expression of said protein, isoforms, analogs, fragments or derivatives, effecting post-translational modification, as necessary, for obtaining said protein, isoform, analogs, fragments or derivatives and isolating said expressed protein, isoforms, analogs, fragments or derivatives.

In a further aspect, the invention provides antibodies or active fragments or derivatives thereof, specific for the above TRAF-binding proteins, analogs, isoforms, fragments or derivatives thereof or specific for the NIK protein, isoform, analog, fragment or derivative thereof noted above.

In a different aspect, the invention provides the following screening methods:

(i) A method for screening of a ligand capable of binding to a protein according to the invention, as noted above, including isoforms, analogs, fragments or derivatives thereof, comprising contacting an affinity chromatography matrix to which said protein, isoform, analog, fragment or derivative is attached with a cell extract whereby the ligand is bound to said matrix, and eluting, isolating and analyzing said ligand.

(ii) A method for screening of a DNA sequence coding for a ligand capable of binding to a protein, isoform, analog, fragment or derivative according to the invention as noted above, comprising applying the yeast two-hybrid procedure in which a sequence encoding said protein, isoform, analog, derivative or fragment is carried by one hybrid vector and sequences from a cDNA or genomic DNA library are carried by the second hybrid vector, transforming yeast host cells with said vectors, isolating the positively transformed cells, and extracting said second hybrid vector to obtain a sequence encoding said ligand.

Similarly, there is also provided a method for isolating and identifying proteins, isoforms, analogs, fragments according to the invention noted above, capable of binding directly to TRAF2, comprising applying the yeast two-hybrid procedure in which a sequence encoding said TRAF2 is carried by one hybrid vector and sequence from a cDNA or genomic DNA library is carried by the second hybrid vector, the vectors then being used to transform yeast host cells and the positive transformed cells being isolated, followed by extraction of the said second hybrid vector to obtain a sequence encoding a protein which binds to said TRAF2.

In yet another aspect of the invention there is provided a method for the modulation or mediation in cells of the activity of NF- κ B or any other intracellular signaling activity modulated or mediated by TRAF2 or by other molecules to which a protein, isoform, analog, fragment or derivative thereof of the invention as noted above, said method comprising treating said cells by introducing into said cells one or more of said protein, isoform, analog, fragment or derivative thereof in a form suitable for intracellular introduction thereof, or introducing into said cells a DNA sequence encoding said one or more protein, isoform, analog, fragment or derivative thereof in the form of a suitable vector carrying said sequence, said vector being capable of effecting the insertion of said sequence into said cells in a way that said sequence is expressed in said cells.

Embodiments of this above method for modulation/mediation in cells of the activity of NF- κ B or any other intracellular signaling activity modulated or mediated by TRAF2 or other molecules include :

(i) A method as above, wherein said treating of cells comprises introducing into said cells a DNA sequence encoding said protein, isoform, fragment, analog or derivative in the form of a suitable vector carrying said sequence, said vector being capable of effecting the

insertion of said sequence into said cells in a way that said sequence is expressed in said cells.

(ii) A method as above wherein said treating of said cells is by transfection of said cells with a recombinant animal virus vector comprising the steps of:

5 (a) constructing a recombinant animal virus vector carrying a sequence encoding a viral surface protein (ligand) that is capable of binding to a specific cell surface receptor on the surface of said cells to be treated and a second sequence encoding a protein selected from the said protein, isoforms, analogs, fragments and derivatives according to the invention, that when expressed in said cells is capable of modulating/mediating the activity
10 of NF- κ B or any other intracellular signaling activity modulated/mediated by TRAF2 or other said molecules; and

(b) infecting said cells with said vector of (a).

Likewise, the present invention also provides a method for modulating TRAF2 modulated/mediated effect on cells comprising treating said cells with the antibodies or
15 active fragments or derivatives thereof, according to the invention as noted above, said treating being by application of a suitable composition containing said antibodies, active fragments or derivatives thereof to said cells, wherein when the TRAF2-binding protein or portions thereof of said cells are exposed on the extracellular surface, said composition is formulated for extracellular application, and when said TRAF2-binding proteins are
20 intracellular said composition is formulated for intracellular application.

Other methods of the invention for modulating the TRAF2 modulated/mediated effect on cells include:

(i) A method comprising treating said cells with an oligonucleotide sequence encoding an antisense sequence for at least part of the DNA sequence encoding a TRAF2-
25 binding protein, this DNA sequence being any of the above mentioned ones of the invention, said oligonucleotide sequence being capable of blocking the expression of the TRAF2-binding protein.

(ii) A method as in (i) above wherein said oligonucleotide sequence is introduced to said cells via a recombinant virus as noted above, wherein said second sequence of said
30 virus encodes said oligonucleotide sequence.

(iii) A method comprising applying the ribozyme procedure in which a vector encoding a ribozyme sequence capable of interacting with a cellular mRNA sequence

encoding a TRAF2-binding protein, isoform, analog, fragment or derivative of the invention noted above, is introduced into said cells in a form that permits expression of said ribozyme sequence in said cells, and wherein when said ribozyme sequence is expressed in said cells it interacts with said cellular mRNA sequence and cleaves said mRNA sequence resulting in the inhibition of expression of said TRAF2-binding protein in said cells.

It should be noted that for all the above methods of the invention the protein of the invention as indicated, can be specifically NIK or at least one of the NIK isoforms, analogs, fragments and derivatives thereof.

In the above methods and embodiments thereof of the invention there is included also a method for the prevention or treatment of a pathological condition associated with NF- κ B induction or with any other activity mediated by TRAF2 or by other molecules to which a protein, isoform, analog, fragment or derivative, according to the invention, binds, said method comprising administering to a patient in need an effective amount of a protein, isoform, analog, fragment or derivative, according to the invention, or a DNA molecule coding therefor, or a molecule capable of disrupting the interaction of said protein, isoform, analog, fragment or derivative, with TRAF2 or any other molecule to which said protein, isoform, analog, fragment or derivative binds. In this method of the invention, said protein of the invention administered to the patient in need can be specifically the protein encoded by clone 10, NIK, an isoform, analog, derivative or fragment of NIK, or a DNA molecule coding therefor. The protein encoded by clone 10 acts to inhibit NF- κ B induction, as do other fragments of NIK, while NIK induces NF- κ B activation.

In an additional aspect of the invention there is provided a pharmaceutical composition for the modulation of the TRAF2 modulated/mediated effect on cells comprising, as active ingredient at least one of the TRAF2-binding proteins, according to the invention, its biologically active fragments, analogs, derivatives or mixtures thereof.

Other pharmaceutical compositions or embodiments thereof according to the invention include :

(i) A pharmaceutical composition for modulating the TRAF2 modulated/mediated effect on cells comprising, as active ingredient, a recombinant animal virus vector encoding a protein capable of binding a cell surface receptor and encoding at least one TRAF2-binding protein, isoform, active fragments or analogs, according to the invention.

(ii) A pharmaceutical composition for modulating the TRAF2 modulated/mediated effect on cells comprising as active ingredient, an oligonucleotide sequence encoding an anti-sense sequence of the TRAF2-binding protein mRNA sequence according to the invention.

5 A further embodiment of the above pharmaceutical composition is specifically a pharmaceutical composition for the prevention or treatment of a pathological condition associated with NF- κ B induction or with any other activity mediated by TRAF2 or by other molecules to which a protein, analog, isoform, fragment or derivative, according to the invention binds, said composition comprising an effective amount of a protein, analog,
10 isoform, fragment or derivative, according to the invention or a DNA molecule coding therefor, or a molecule capable of disrupting the interaction of said protein, analog, isoform, fragment or derivative, with TRAF2 or any other molecule to which said protein, analog, isoform, fragment or derivative, binds. In a yet further specific embodiment said pharmaceutical composition comprising an effective amount of the protein encoded by
15 clone 10, NIK, an isoform, analog, derivative or fragment of NIK, or a DNA molecule coding therefor.

In yet another specific embodiment, the invention provides a pharmaceutical composition for the prevention or treatment of a pathological condition associated with NF- κ B induction or with any other activity mediated by TRAF2 or by other molecules to
20 which the protein NIK binds, said composition comprising a molecule capable of interfering with the protein kinase activity of NIK. In this composition, the interfering molecule may be an effective amount of NIK mutated in active site residues, this mutated NIK serving to interfere with native NIK, in particular, the kinase activity of NIK.

One known condition associated with NF- κ B induction (abnormal) is AIDS, others
25 are e.g. autoimmune diseases, as well as tumors.

Still further aspects and embodiments of the invention are :

(i) A method for identifying and producing a ligand capable of modulating the cellular activity modulated/mediated by TRAF2 comprising :

a) screening for a ligand capable of binding to a polypeptide comprising at
30 least a portion of TRAF2 having the amino acid residues 221-501 of TRAF2;

b) identifying and characterizing a ligand, other than TRAF2 or portions of a receptor of the TNF/NGF receptor family, found by said screening step to be capable of said binding; and

c) producing said ligand in substantially isolated and purified form.

5 (ii) A method for identifying and producing a ligand capable of modulating the cellular activity modulated/mediated by a protein, isoform, analog, fragment or derivative, according to the invention, comprising :

a) screening for a ligand capable of binding to a polypeptide comprising at least a portion of the NIK sequence depicted in Fig. 6;

10 b) identifying and characterizing a ligand, other than TRAF2 or portions of a receptor of the TNF/NGF receptor family, found by said screening step to be capable of said binding; and

c) producing said ligand in substantially isolated and purified form.

15 (iii) A method for identifying and producing a ligand capable of modulating the cellular activity modulated/mediated by NIK comprising :

a) screening for a ligand capable of binding to a polypeptide comprising at least a portion of the NIK sequence depicted in Fig. 6;

20 b) identifying and characterizing a ligand, other than TRAF2 or portions of a receptor of the TNF/NGF receptor family, found by said screening step to be capable of said binding; and

c) producing said ligand in substantially isolated and purified form.

(iv) A method for identifying and producing a ligand capable of directly or indirectly modulating the cellular activity modulated/mediated by NIK comprising :

25 a) screening for a molecule capable of modulating activities modulated/mediated by NIK;

b) identifying and characterizing said molecule; and

c) producing said molecule in substantially isolated and purified form.

30 (v) A method for identifying and producing a molecule capable of directly or indirectly modulating the cellular activity modulated/mediated by a protein, isoform, analog, fragment or derivative of the invention, comprising :

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a) screening for a molecule capable of modulating activities modulated/mediated by a protein, isoform, analog, fragment or derivative according to the invention;

b) identifying and characterizing said molecule; and

c) producing said molecule in substantially isolated and purified form.

Other aspects and embodiments of the present invention are also provided as arising from the following detailed description of the invention.

It should be noted that, where used throughout, the following terms :
"modulation/mediation of the TRAF (or TRAF2) effect on cells" and any other such
"modulation/mediation" mentioned in the specification are understood to encompass *in vitro* as well as *in vivo* treatment and, in addition, also to encompass inhibition or enhancement/augmentation.

Brief Description of the Drawings

Fig. 1 shows a diagrammatic illustration of the structure of the TRAF2 molecule.

Fig. 2a-b shows schematic diagrams illustrating some of the proteins involved in NF- κ B activation, including the new TRAF-binding proteins of the present invention (e.g. NIK), in which (a) is a partial scheme and (b) is a more complete scheme;

Figs. 3a-b show the nucleotide sequence of the 5' end of clone 9 (a) and the deduced amino acid sequence encoded thereby (b);

Fig. 4 shows the nucleotide sequence of clone 10;

Figs. 5a-b show the nucleotide sequence of clone 15 (a) and the deduced amino acid sequence encoded thereby (b);

Fig. 6 shows the nucleotide sequence and the deduced amino acid sequence of NIK;

Fig. 7 shows an alignment of the sequence of protein NIK with the sequence of the mouse protein kinase mMEKK (mouse MAPK or ERK Kinase Kinase) and a number of other kinases. The regions corresponding to the conserved motifs I to XI in protein kinases are marked.

Detailed Description of the Invention

The present invention relates to DNA sequences encoding proteins capable of binding to a tumor necrosis factor receptor-associated factor (TRAF) molecule, and the proteins encoded thereby.

5 In a preferred embodiment, the present invention concerns cDNA sequences herein designated clone 9, clone 10 and clone 15 (depicted in Figs. 3a, 4 and 5a, respectively), which encode for proteins capable of binding to TRAF2, and the proteins encoded by those DNA sequences.

10 In a further preferred embodiment the invention relates to the DNA sequence encoding the NIK protein, and the NIK protein itself.

The DNA and the deduced amino acid sequences mentioned above represent new sequences; they do not appear in the 'GENEBANK' or 'PROTEIN BANK' data banks of DNA or amino acid sequences.

15 Within the scope of the present invention are also fragments of the above mentioned DNA sequences and DNA sequences capable of hybridization to those sequences or part of them, under moderately stringent conditions, provided they encode a biologically active protein or polypeptide capable of binding to at least the 222-501 amino acid sequence of TRAF2.

20 The present invention also concerns a DNA sequence which is degenerate as a result of the genetic code to the above mentioned DNA sequences and which encodes a biologically active protein or polypeptide capable of binding to at least the 222-501 amino acid sequence of TRAF2.

As regards TRAF2, it should be noted that several members of the TNF/NGF receptor family activate the transcription factor NF- κ B by direct or indirect association with TRAF2, which is thus an adaptor protein for these receptors and may thus also be considered as a modulator/mediator of the induction of NF- κ B activation activity of these TNF/NGF receptors (see the scheme in Fig. 2b). Another receptor, the IL-1 receptor activates NF- κ B independently of TRAF2. One of the embodiments of a preferred TRAF2-binding protein in accordance with the present invention is the NIK protein, which binds NIK in a very specific way and stimulates NF- κ B activity. NIK is a serine/threonine kinase having sequence similarity with several MAPKK kinases (see Examples below). NIK analogs or muteins produced in accordance with the present invention (see Examples)

which lack the kinase activity of NIK fail to stimulate NF- κ B activation, when these analogs/mutins are expressed in cells. Further, such NIK analogs/mutins when expressed in cells also block NF- κ B induction by TNF as well as by other inducing agents such as the bacterial endotoxin LPS, forbol myristate acetate (a protein kinase C activator), and the HTLV-1 protein TAX. TNF induction of NF- κ B activity is via either of the two TNF receptors (p55 and p75 TNF receptors) and hence it appears that the NIK mutin/analogs block induction of NF- κ B activation via these receptors. Likewise, TNF and the FAS/APO1 receptor ligand may also induce NF- κ B activity via a related receptor, the FAS/APO1 receptor, which induction is also blocked by NIK mutins/analogs. Moreover, the above receptors have adaptor proteins TRADD, RIP and MORT1 which can all also induce NF- κ B activity, but which induction is also blocked by NIK mutins/analogs. In addition, such NIK mutins/analogs also blocked NF- κ B induction by IL-1 (functioning via the IL-1 receptor). Accordingly, it arises that NIK participates in an NF- κ B-inducing cascade that is common to receptors of the TNF/NGF family and to the IL-1 receptor. NIK also appears to act in a direct way in inducing NF- κ B activation possibly by enhancing I- κ B phosphorylation directly. This arises from the present observations that the above NIK analogs/mutins lacking kinase activity (also called dominant-negative mutants) when expressed in cells did not effect in any manner the TNF-induced activation of Jun kinase, indicating that NIK acts specifically to enhance phosphorylation of I- κ B without affecting the MAP kinase involved in Jun phosphorylation.

Thus, the present invention concerns the DNA sequences encoding biologically active TRAF-binding proteins, e.g. TRAF2-binding proteins, such as, for example, NIK, as well as analogs, fragments and derivatives thereof, and the analogs, fragments and derivatives of the proteins encoded thereby. The preparation of such analogs, fragments and derivatives is by standard procedures (see for example, Sambrook et al., 1989) in which in the DNA encoding sequences, one or more codons may be deleted, added or substituted by another, to yield encoded analogs having at least a one amino acid residue change with respect to the native protein. Acceptable analogs are those which retain at least the capability of binding to TRAF2 with or without mediating any other binding or enzymatic activity, e.g. analogs which bind TRAF2 but do not signal, i.e. do not bind to a further downstream protein or other factor, or do not catalyze a signal-dependent reaction. In such a way analogs can be produced which have a so-called dominant-negative effect,

namely, an analog which is defective either in binding to TRAF2, or in subsequent signaling following such binding as noted above. Such analogs can be used, for example, to inhibit the CD40, p55 TNF and p75 TNF (FAS/APO1 and other related receptor effects, as well as effected mediated by various receptor associated proteins (adaptors) as noted above, by competing with the natural TRAF2-binding proteins. Likewise, so-called dominant-positive analogs may be produced which would serve to enhance the TRAF2 effect. These would have the same or better TRAF2-binding properties and the same or better signaling properties of the natural TRAF2-binding proteins. In an analogous fashion, biologically active fragments of the clones of the invention may be prepared as noted above with respect to the preparation of the analogs. Suitable fragments of the DNA sequences of the invention are those which encode a protein or polypeptide retaining the TRAF2 binding capability or which can mediate any other binding or enzymatic activity as noted above. Accordingly, fragments of the encoded proteins of the invention can be prepared which have a dominant-negative or a dominant-positive effect as noted above with respect to the analogs. Similarly, derivatives may be prepared by standard modifications of the side groups of one or more amino acid residues of the proteins, their analogs or fragments, or by conjugation of the proteins, their analogs or fragments, to another molecule e.g. an antibody, enzyme, receptor, etc., as are well known in the art.

Of the above DNA sequences of the invention which encode a TRAF-binding protein, (e.g. TRAF2-binding protein, such as for example, NIK) isoform, analog, fragment or derivative, there is also included, as an embodiment of the invention, DNA sequences capable of hybridizing with a cDNA sequence derived from the coding region of a native TRAF-binding protein, in which such hybridization is performed under moderately stringent conditions, and which hybridizable DNA sequences encode a biologically active TRAF-binding protein. These hybridizable DNA sequences therefore include DNA sequences which have a relatively high homology to the native TRAF-binding proteins cDNA sequence, (e.g. TRAF2-binding protein cDNA sequence, such as, for example the NIK cDNA sequence) and as such represent TRAF-binding protein-like sequences which may be, for example, naturally-derived sequences encoding the various TRAF-binding protein isoforms, or naturally-occurring sequences encoding proteins belonging to a group of TRAF-binding protein-like sequences encoding a protein having the activity of TRAF-binding proteins (e.g. TRAF2-binding proteins, such as, for example, NIK). Further, these

sequences may also, for example, include non-naturally occurring, synthetically produced sequences, that are similar to the native TRAF-binding protein cDNA sequence but incorporate a number of desired modifications. Such synthetic sequences therefore include all of the possible sequences encoding analogs, fragments and derivatives of TRAF-binding proteins (e.g. TRAF2-binding proteins, such as, for example NIK), all of which have the activity of TRAF-binding proteins.

To obtain the various above noted naturally occurring TRAF-binding protein-like sequences, standard procedures of screening and isolation of naturally-derived DNA or RNA samples from various tissues may be employed using the natural TRAF-binding protein cDNA or portion thereof as probe (see for example standard procedures set forth in Sambrook et al., 1989).

Likewise, to prepare the above noted various synthetic TRAF-binding protein-like sequences encoding analogs, fragments or derivatives of TRAF-binding proteins (e.g. TRAF2-binding proteins, such as, for example NIK), a number of standard procedures may be used as are detailed herein below concerning the preparation of such analogs, fragments and derivatives.

A polypeptide or protein "substantially corresponding" to TRAF-binding protein includes not only TRAF-binding protein but also polypeptides or proteins that are analogs of TRAF-binding protein.

Analogues that substantially correspond to TRAF-binding protein are those polypeptides in which one or more amino acid of the TRAF-binding protein's amino acid sequence has been replaced with another amino acid, deleted and/or inserted, provided that the resulting protein exhibits substantially the same or higher biological activity as the TRAF-binding protein to which it corresponds.

In order to substantially correspond to TRAF-binding protein, the changes in the sequence of TRAF-binding proteins, such as isoforms are generally relatively minor. Although the number of changes may be more than ten, preferably there are no more than ten changes, more preferably no more than five, and most preferably no more than three such changes. While any technique can be used to find potentially biologically active proteins which substantially correspond to TRAF-binding proteins, one such technique is the use of conventional mutagenesis techniques on the DNA encoding the protein, resulting in a few modifications. The proteins expressed by such clones can then be screened for

their ability to bind to TRAF proteins (e.g. TRAF2) and to modulate TRAF protein. (e.g. TRAF2) activity in modulation/mediation of the intracellular pathways noted above.

"Conservative" changes are those changes which would not be expected to change the activity of the protein and are usually the first to be screened as these would not be expected to substantially change the size, charge or configuration of the protein and thus would not be expected to change the biological properties thereof.

Conservative substitutions of TRAF-binding proteins include an analog wherein at least one amino acid residue in the polypeptide has been conservatively replaced by a different amino acid. Such substitutions preferably are made in accordance with the following list as presented in Table IA, which substitutions may be determined by routine experimentation to provide modified structural and functional properties of a synthesized polypeptide molecule while maintaining the biological activity characteristic of TRAF-binding protein.

Table IA

	<u>Original</u> <u>Residue</u>	<u>Exemplary</u> <u>Substitution</u>
5	Ala	Gly,Ser
	Arg	Lys
	Asn	Gln,His
	Asp	Glu
10	Cys	Ser
	Gln	Asn
	Glu	Asp
	Gly	Ala;Pro
	His	Asn;Gln
15	Ile	Leu;Val
	Leu	Ile;Val
	Lys	Arg;Gln;Glu
	Met	Leu;Tyr,Ile
	Phe	Met;Leu;Tyr
20	Ser	Thr
	Thr	Ser
	Trp	Tyr
	Tyr	Trp;Phe
	Val	Ile;Leu

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Alternatively, another group of substitutions of TRAF-binding protein are those in which at least one amino acid residue in the polypeptide has been removed and a different residue inserted in its place according to the following Table IB. The types of substitutions which may be made in the polypeptide may be based on analysis of the frequencies of amino acid changes between a homologous protein of different species, such as those presented in Table 1-2 of Schulz et al., G.E., Principles of Protein Structure Springer-

Verlag, New York, NY, 1798, and Figs. 3-9 of Creighton, *T.E.*, *Proteins: Structure and Molecular Properties*, W.H. Freeman & Co., San Francisco, CA 1983. Based on such an analysis, alternative conservative substitutions are defined herein as exchanges within one of the following five groups:

TABLE IB

1. Small aliphatic, nonpolar or slightly polar residues: Ala, Ser, Thr (Pro, Gly);
2. Polar negatively charged residues and their amides: Asp, Asn, Glu, Gln;
3. Polar, positively charged residues: His, Arg, Lys;
4. Large aliphatic nonpolar residues: Met, Leu, Ile, Val (Cys); and
5. Large aromatic residues: Phe, Tyr, Trp.

The three amino acid residues in parentheses above have special roles in protein architecture. Gly is the only residue lacking any side chain and thus imparts flexibility to the chain. This however tends to promote the formation of secondary structure other than α -helical. Pro, because of its unusual geometry, tightly constrains the chain and generally tends to promote β -turn-like structures, although in some cases Cys can be capable of participating in disulfide bond formation which is important in protein folding. Note that Schulz *et al.*, *supra*, would merge Groups 1 and 2, above. Note also that Tyr, because of its hydrogen bonding potential, has significant kinship with Ser, and Thr, etc.

Conservative amino acid substitutions according to the present invention, e.g., as presented above, are known in the art and would be expected to maintain biological and structural properties of the polypeptide after amino acid substitution. Most deletions and substitutions according to the present invention are those which do not produce radical changes in the characteristics of the protein or polypeptide molecule. "Characteristics" is defined in a non-inclusive manner to define both changes in secondary structure, e.g. α -

helix or β -sheet, as well as changes in biological activity, e.g., binding to TRAF proteins and/or mediation of TRAF proteins' effect on cell death.

Examples of production of amino acid substitutions in proteins which can be used for obtaining analogs of TRAF-binding proteins for use in the present invention include any known method steps, such as presented in U.S. patent RE 33,653, 4,959,314, 4,588,585 and 4,737,462, to Mark et al.; 5,116,943 to Koths et al., 4,965,195 to Namen et al.; 4,879,111 to Chong et al.; and 5,017,691 to Lee et al.; and lysine substituted proteins presented in U.S. patent No. 4,904,584 (Shaw et al.).

Besides conservative substitutions discussed above which would not significantly change the activity of TRAF-binding protein, either conservative substitutions or less conservative and more random changes, which lead to an increase in biological activity of the analogs of TRAF-binding proteins, are intended to be within the scope of the invention.

When the exact effect of the substitution or deletion is to be confirmed, one skilled in the art will appreciate that the effect of the substitution(s), deletion(s), etc., will be evaluated by routine binding and cell death assays. Screening using such a standard test does not involve undue experimentation.

At the genetic level, these analogs are generally prepared by site-directed mutagenesis of nucleotides in the DNA encoding the TRAF-binding protein, thereby producing DNA encoding the analog, and thereafter synthesizing the DNA and expressing the polypeptide in recombinant cell culture. The analogs typically exhibit the same or increased qualitative biological activity as the naturally occurring protein, Ausubel *et al.*, Current Protocols in Molecular Biology, Greene Publications and Wiley Interscience, New York, NY, 1987-1995; Sambrook *et al.*, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1989.

Preparation of a TRAF-binding protein in accordance herewith, or an alternative nucleotide sequence encoding the same polypeptide but differing from the natural sequence due to changes permitted by the known degeneracy of the genetic code, can be achieved by site-specific mutagenesis of DNA that encodes an earlier prepared analog or a native version of a TRAF-binding protein. Site-specific mutagenesis allows the production of analogs through the use of specific oligonucleotide sequences that encode the DNA sequence of the desired mutation, as well as a sufficient number of adjacent nucleotides, to provide a primer sequence of sufficient size and sequence complexity to form a stable

duplex on both sides of the deletion junction being traversed. Typically, a primer of about 20 to 25 nucleotides in length is preferred, with about 5 to 10 complementing nucleotides on each side of the sequence being altered. In general, the technique of site-specific mutagenesis is well known in the art, as exemplified by publications such as Adelman *et al.*, *DNA* 2:183 (1983), the disclosure of which is incorporated herein by reference.

As will be appreciated, the site-specific mutagenesis technique typically employs a phage vector that exists in both a single-stranded and double-stranded form. Typical vectors useful in site-directed mutagenesis include vectors such as the M13 phage, for example, as disclosed by Messing *et al.*, *Third Cleveland Symposium on Macromolecules and Recombinant DNA*, Editor A. Walton, Elsevier, Amsterdam (1981), the disclosure of which is incorporated herein by reference. These phages are readily available commercially and their use is generally well known to those skilled in the art. Alternatively, plasmid vectors that contain a single-stranded phage origin of replication (Veira *et al.*, *Meth. Enzymol.* 153:3, 1987) may be employed to obtain single-stranded DNA.

In general, site-directed mutagenesis in accordance herewith is performed by first obtaining a single-stranded vector that includes within its sequence a DNA sequence that encodes the relevant polypeptide. An oligonucleotide primer bearing the desired mutated sequence is prepared synthetically by automated DNA/oligonucleotide synthesis. This primer is then annealed with the single-stranded protein-sequence-containing vector, and subjected to DNA-polymerizing enzymes such as *E. coli* polymerase I Klenow fragment, to complete the synthesis of the mutation-bearing strand. Thus, a mutated sequence and the second strand bears the desired mutation. This heteroduplex vector is then used to transform appropriate cells, such as *E. coli* JM101 cells, and clones are selected that include recombinant vectors bearing the mutated sequence arrangement.

After such a clone is selected, the mutated TRAF-binding protein sequence may be removed and placed in an appropriate vector, generally a transfer or expression vector of the type that may be employed for transfection of an appropriate host.

Accordingly, gene or nucleic acid encoding for a TRAF-binding protein can also be detected, obtained and/or modified, *in vitro*, *in situ* and/or *in vivo*, by the use of known DNA or RNA amplification techniques, such as PCR and chemical oligonucleotide synthesis. PCR allows for the amplification (increase in number) of specific DNA sequences by repeated DNA polymerase reactions. This reaction can be used as a

replacement for cloning; all that is required is a knowledge of the nucleic acid sequence.

In order to carry out PCR, primers are designed which are complementary to the sequence of interest. The primers are then generated by automated DNA synthesis. Because primers can be designed to hybridize to any part of the gene, conditions can be created such that mismatches in complementary base pairing can be tolerated. Amplification of these mismatched regions can lead to the synthesis of a mutagenized product resulting in the generation of a peptide with new properties (i.e., site directed mutagenesis). See also, e.g., Ausubel, *supra*, Ch. 16. Also, by coupling complementary DNA (cDNA) synthesis, using reverse transcriptase, with PCR, RNA can be used as the starting material for the synthesis of the extracellular domain of a prolactin receptor without cloning.

Furthermore, PCR primers can be designed to incorporate new restriction sites or other features such as termination codons at the ends of the gene segment to be amplified. This placement of restriction sites at the 5' and 3' ends of the amplified gene sequence allows for gene segments encoding TRAF-binding protein or a fragment thereof to be custom designed for ligation other sequences and/or cloning sites in vectors.

PCR and other methods of amplification of RNA and/or DNA are well known in the art and can be used according to the present invention without undue experimentation, based on the teaching and guidance presented herein. Known methods of DNA or RNA amplification include, but are not limited to polymerase chain reaction (PCR) and related amplification processes (see, e.g., U.S. patent Nos. 4,683,195, 4,683,202, 4,800,159, 4,965,188, to Mullis *et al.*; 4,795,699 and 4,921,794 to Tabor *et al.*; 5,142,033 to Innis; 5,122,464 to Wilson *et al.*; 5,091,310 to Innis; 5,066,584 to Gyllenstein *et al.*; 4,889,818 to Gelfand *et al.*; 4,994,370 to Silver *et al.*; 4,766,067 to Biswas; 4,656,134 to Ringold; and Innis *et al.*, eds., *PCR Protocols: A Guide to Method and Applications*) and RNA mediated amplification which uses anti-sense RNA to the target sequence as a template for double stranded DNA synthesis (U.S. patent No. 5,130,238 to Malek *et al.*, with the tradename NASBA); and immuno-PCR which combines the use of DNA amplification with antibody labeling (Ruzicka *et al.*, *Science* 260:487 (1993); Sano *et al.*, *Science* 258:120 (1992); Sano *et al.*, *Biotechniques* 9:1378 (1991)), the entire contents of which patents and reference are entirely incorporated herein by reference.

In an analogous fashion, biologically active fragments of TRAF-binding proteins (e.g. those of any of the TRAF2-binding proteins, such as, for example NIK) or its

isoforms) may be prepared as noted above with respect to the analogs of TRAF-binding proteins. Suitable fragments of TRAF-binding proteins are those which retain the TRAF-binding protein capability and which can mediate the biological activity of TRAF proteins or other proteins associated with TRAF proteins directly or indirectly. Accordingly, TRAF-binding protein fragments can be prepared which have a dominant-negative or a dominant-positive effect as noted above with respect to the analogs. It should be noted that these fragments represent a special class of the analogs of the invention, namely, they are defined portions of TRAF-binding proteins derived from the full TRAF-binding protein sequence (e.g., from that of any one of the TRAF2-binding proteins, such as, for example NIK or its isoforms), each such portion or fragment having any of the above-noted desired activities. Such fragment may be, e.g., a peptide.

Similarly, derivatives may be prepared by standard modifications of the side groups of one or more amino acid residues of the TRAF-binding protein, its analogs or fragments, or by conjugation of the TRAF-binding protein, its analogs or fragments, to another molecule e.g. an antibody, enzyme, receptor, etc., as are well known in the art. Accordingly, "derivatives" as used herein covers derivatives which may be prepared from the functional groups which occur as side chains on the residues or the N- or C-terminal groups, by means known in the art, and are included in the invention. Derivatives may have chemical moieties such as carbohydrate or phosphate residues, provided such a fraction has the same or higher biological activity as TRAF-binding proteins.

For example, derivatives may include aliphatic esters of the carboxyl groups, amides of the carboxyl groups by reaction with ammonia or with primary or secondary amines, N-acyl derivatives or free amino groups of the amino acid residues formed with acyl moieties (e.g., alkanoyl or carbocyclic aryl groups) or O-acyl derivatives of free hydroxyl group (for example that of seryl or threonyl residues) formed with acyl moieties.

The term "derivatives" is intended to include only those derivatives that do not change one amino acid to another of the twenty commonly occurring natural amino acids.

A TRAF-binding protein is a protein or polypeptide, i.e. a sequence of amino acid residues. A polypeptide consisting of a larger sequence which includes the entire sequence of a TRAF-binding protein, in accordance with the definitions herein, is intended to be included within the scope of such a polypeptide as long as the additions do not affect the basic and novel characteristics of the invention, i.e., if they either retain or increase the

biological activity of TRAF-binding protein or can be cleaved to leave a protein or polypeptide having the biological activity of TRAF-binding protein. Thus, for example, the present invention is intended to include fusion proteins of TRAF-binding protein with other amino acids or peptides.

As mentioned above, it should be understood that the above 'TRAF-binding' proteins of the invention are any proteins which may bind and mediate/modulate the activity of any TRAF protein intracellularly. Particular examples are the TRAF2-binding proteins which can modulate or mediate the TRAF2-associated intracellular signaling activity, as mentioned above, especially as concerns TRAF2's involvement in inducing NF- κ B activity, in particular, following the interaction between TRAF2 and various members of the TNF/NGF receptor family and/or their associated adaptor proteins as detailed above and below. A specific example of such TRAF2-binding proteins is the NIK protein and its various analogs, fragments, etc. (see Examples) which appears to bind TRAF2 very specifically and to have a direct action in inducing NF- κ B activity, with various NIK dominant-negative analogs/mutins blocking this activity.

All the above mentioned modifications are in the scope of the invention provided they preserved the ability of the encoded proteins or polypeptides or their analogs and derivatives thereof, to bind at least the 222-501 amino acid sequence of TRAF2.

All the proteins and polypeptides of the invention by virtue of their capability to bind to TRAF2, are considered as mediators or modulators of TRAF2 signaling. As such, said molecules of the invention have a role in, for example, the signaling process in which the binding of TRAF2 ligand to CD30, CD40, lymphotoxin beta (LT- β) receptor, p55 or p75 TNF receptors, as well as the other receptors and adaptor proteins noted herein above, leads to activation of the transcription factor NF- κ B. Particularly interesting is protein NIK and a partial NIK protein, encoded by clone 10 of the invention; a detailed sequence analysis of NIK and this clone-10-encoded protein (originally termed NMPI) disclosed encoded amino acid sequences corresponding to I - XI conserved motifs characteristic to Ser/Thr protein kinases, thus assigning a function to this protein.

The new clones proteins, their analogs, fragments and derivatives have a number of possible uses, for example:

(i) They may be used to mimic or enhance NF κ B activity, the function of TRAF2 and the receptors to which they bind, in situations where an enhanced function is desired

such as in anti-tumor or immuno-stimulatory applications where the TRAF2- induced effects are desired. In this case the proteins of the invention, their analogs, fragments or derivatives, which enhance the TRAF2 or receptors effects, may be introduced to the cells by standard procedures known per se. For example, as the proteins encoded by the DNA clones of the invention are intracellular and they should be introduced only into the cells where the TRAF2 effect is desired, a system for specific introduction of these proteins into the cells is necessary. One way of doing this is by creating a recombinant animal virus e.g. one derived from Vaccinia, to the DNA of which the following two genes will be introduced: the gene encoding a ligand that binds to cell surface proteins specifically expressed by the cells e.g. ones such as the AIDs (HIV) virus gp120 protein which binds specifically to some cells (CD4 lymphocytes and related leukemias) or any other ligand that binds specifically to cells carrying a receptor that binds TRAF2, such that the recombinant virus vector will be capable of binding such cells; and the gene encoding the proteins of the invention. Thus, expression of the cell-surface-binding protein on the surface of the virus will target the virus specifically to the tumor cell or other receptor- carrying cell, following which the proteins encoding sequences will be introduced into the cells via the virus, and once expressed in the cells will result in enhancement of the receptor or TRAF2 effect leading to a desired immuno-stimulatory effect in these cells. Construction of such recombinant animal virus is by standard procedures (see for example, Sambrook et al., 1989). Another possibility is to introduce the sequences of the encoded proteins in the form of oligonucleotides which can be absorbed by the cells and expressed therein.

(ii) They may be used to inhibit the NF κ B activity, the effects of TRAF2 or of the receptor that binds it, e.g. in cases such as tissue damage as in AIDS, septic shock or graft-vs.-host rejection, in which it is desired to block the induced intracellular signaling. In this situation it is possible, for example, to introduce into the cells, by standard procedures, oligonucleotides having the anti-sense coding sequence for the proteins of the invention, which would effectively block the translation of mRNAs encoding the proteins and thereby block their expression and lead to the inhibition of the undesired effect. Alternatively, other oligonucleotides may be used; oligonucleotides that preserved their ability to bind to TRAF2 in a way that interferes with the binding of other molecules to this protein, while at the same time do not mediate any activation or modulation of this molecule. Having these characteristics, said molecules can disrupt the interaction of TRAF2 with its natural ligand,

therefor acting as inhibitors capable of abolishing effects mediated by TRAF2, such as NF- κ B activation, for example. Such oligonucleotides may be introduced into the cells using the above recombinant virus approach, the second sequence carried by the virus being the oligonucleotide sequence.

5 Another possibility is to use antibodies specific for the proteins of the invention to inhibit their intracellular signaling activity.

Yet another way of inhibiting the undesired effect is by the recently developed ribozyme approach. Ribozymes are catalytic RNA molecules that specifically cleave RNAs. Ribozymes may be engineered to cleave target RNAs of choice, e.g. the mRNAs encoding
10 the proteins of the invention. Such ribozymes would have a sequence specific for the mRNA of the proteins and would be capable of interacting therewith (complementary binding) followed by cleavage of the mRNA, resulting in a decrease (or complete loss) in the expression of the proteins, the level of decreased expression being dependent upon the level of ribozyme expression in the target cell. To introduce ribozymes into the cells of
15 choice (e.g. those carrying the TRAF2 binding proteins) any suitable vector may be used, e.g. plasmid, animal virus (retrovirus) vectors, that are usually used for this purpose (see also (i) above, where the virus has, as second sequence, a cDNA encoding the ribozyme sequence of choice). (For reviews, methods etc. concerning ribozymes see Chen et al., 1992; Zhao and Pick, 1993).

20 (iii) They may be used to isolate, identify and clone other proteins which are capable of binding to them, e.g. other proteins involved in the intracellular signaling process that are downstream of TRAF2. For example, the DNA sequences encoding the proteins of the invention may be used in the yeast two-hybrid system in which the encoded proteins will be used as "bait" to isolate, clone and identify from cDNA or genomic DNA libraries other
25 sequences ("preys") encoding proteins which can bind to the clones proteins. In the same way, it may also be determined whether the proteins of the present invention can bind to other cellular proteins, e.g. other receptors of the TNF/NGF superfamily of receptors.

(iv) The encoded proteins, their analogs, fragments or derivatives may also be used to isolate, identify and clone other proteins of the same class i.e. those binding to TRAF2
30 or to functionally related proteins, and involved in the intracellular signaling process. In this application the above noted yeast two-hybrid system may be used, or there may be used a

recently developed system employing non- stringent Southern hybridization followed by PCR cloning (Wilks et al., 1989).

(v) Yet another approach to utilize the encoded proteins of the invention, their analogs, fragments or derivatives is to use them in methods of affinity chromatography to isolate and identify other proteins or factors to which they are capable of binding, e.g., proteins related to TRAF2 or other proteins or factors involved in the intracellular signaling process. In this application, the proteins, their analogs, fragments or derivatives of the present invention, may be individually attached to affinity chromatography matrices and then brought into contact with cell extracts or isolated proteins or factors suspected of being involved in the intracellular signaling process. Following the affinity chromatography procedure, the other proteins or factors which bind to the proteins, their analogs, fragments or derivatives of the invention, can be eluted, isolated and characterized.

(vi) As noted above, the proteins, their analogs, fragments or derivatives of the invention may also be used as immunogens (antigens) to produce specific antibodies thereto. These antibodies may also be used for the purposes of purification of the proteins of the invention either from cell extracts or from transformed cell lines producing them, their analogs or fragments. Further, these antibodies may be used for diagnostic purposes for identifying disorders related to abnormal functioning of the receptor system in which they function, e.g., overactive or underactive TRAF2- induced cellular effects. Thus, should such disorders be related to a malfunctioning intracellular signaling system involving the proteins of the invention, such antibodies would serve as an important diagnostic tool. The term "antibody" is meant to include polyclonal antibodies, monoclonal antibodies (mAbs), chimeric antibodies, anti-idiotypic (anti-Id) antibodies to antibodies that can be labeled in soluble or bound form, as well as fragments thereof, such as, for example, Fab and $F(ab')_2$ fragments lacking the Fc fragment of intact antibody, which are capable of binding antigen.

(vii) The antibodies, including fragments of antibodies, useful in the present invention may be used to quantitatively or qualitatively detect the clones of the invention in a sample, or to detect presence of cells which express the clones of the present invention. This can be accomplished by immunofluorescence techniques employing a fluorescently labeled antibody coupled with light microscopic, flow cytometric, or fluorometric detection.

The antibodies (or fragments thereof) useful in the present invention may be employed histologically, as in immunofluorescence or immunoelectron microscopy, for *in situ* detection of the clones of the present invention. *In situ* detection may be accomplished by removing a histological specimen from a patient, and providing the labeled antibody of the present invention to such a specimen. The antibody (or fragment) is preferably provided by applying or by overlaying the labeled antibody (or fragment) to a biological sample. Through the use of such a procedure, it is possible to determine not only the presence of the clones, but also its distribution on the examined tissue. Using the present invention, those of ordinary skill will readily perceive that any of wide variety of histological methods (such as staining procedures) can be modified in order to achieve such *in situ* detection.

Such assays for the clones of the present invention typically comprises incubating a biological sample, such as a biological fluid, a tissue extract, freshly harvested cells such as lymphocytes or leukocytes, or cells which have been incubated in tissue culture, in the presence of a detectably labeled antibody capable of identifying the encoded proteins, and detecting the antibody by any of a number of techniques well known in the art.

(viii) The encoded proteins of the invention may also be used as indirect modulators of a number of other proteins by virtue of their capability of binding to other intracellular proteins, which other intracellular proteins directly bind yet other intracellular proteins or an intracellular domain of a transmembrane protein.

For the purposes of modulating these other intracellular proteins or the intracellular domains of transmembrane proteins, the proteins of the invention may be introduced into cells in a number of ways as mentioned hereinabove in (ii).

It should also be noted that the isolation, identification and characterization of the proteins of the invention may be performed using any of the well known standard screening procedures. For example, one of these screening procedures, the yeast two-hybrid procedure which was used to identify the proteins of the invention. Likewise other procedures may be employed such as affinity chromatography, DNA hybridization procedures, etc. as are well known in the art, to isolate, identify and characterize the proteins of the invention or to isolate, identify and characterize additional proteins, factors, receptors, etc. which are capable of binding to the proteins of the invention.

Moreover, the proteins found to bind to the proteins of the invention may themselves be employed, in an analogous fashion to the way in which the proteins of the invention were used as noted above and below, to isolate, identify and characterize other proteins, factors, etc. which are capable of binding to the proteins of the invention-binding proteins and which may represent factors involved further downstream in the associated signaling process, or which may have signaling activities of their and hence would represent proteins involved in a distinct signaling process.

The DNA sequences and the encoded proteins of the invention may be produced by any standard recombinant DNA procedure (see for example, Sambrook, et al., 1989) in which suitable eukaryotic or prokaryotic host cells are transformed by appropriate eukaryotic or prokaryotic vectors containing the sequences encoding for the proteins. Accordingly, the present invention also concerns such expression vectors and transformed hosts for the production of the proteins of the invention. As mentioned above, these proteins also include their biologically active analogs, fragments and derivatives, and thus the vectors encoding them also include vectors encoding analogs and fragments of these proteins, and the transformed hosts include those producing such analogs and fragments. The derivatives of these proteins are the derivatives produced by standard modification of the proteins or their analogs or fragments, produced by the transformed hosts.

The present invention also relates to pharmaceutical compositions for modulation of the effects mediated by TRAF2. The pharmaceutical compositions comprising, as an active ingredient, any one or more of the following: (i) one or more of the DNA sequences of the invention, or parts of them, subcloned into an appropriate expression vector; (ii) a protein according to the invention, its biologically active fragments, analogs, derivatives or a mixture thereof; (iii) a recombinant animal virus vector encoding for a protein according to the invention, its biologically active fragments, analogs or derivatives.

The pharmaceutical compositions are applied according to the disease to be treated and in amounts beneficial to the patient, depending on body weight and other considerations, as determined by the physician.

As noted above, one of the specific embodiments of the TRAF-binding proteins of the present invention is the TRAF2-binding protein NIK. Based on the findings in accordance with the present invention that NIK binds specifically to TRAF2 and as such is a mediator/modulator of TRAF2 and can thus mediate/modulate TRAF2's activity in NF-

κB activation and hence its possible role in cell survival pathways in ways that TRAF2 functions independently or in conjunction with other proteins (e.g. p55 TNF and p75 TNF receptors, FAS/APO1 receptor, MORT-1, RIP and TRADD) it is of importance to design drugs which may enhance or inhibit the TRAF2-NIK interaction, as desired. For example, when it is desired to increase the cell cytotoxicity induced by TNF it would be desired to inhibit NF-κB induction, by inhibiting the TRAF2-NIK interaction or by inhibiting TRAF2 and/or NIK specifically. Likewise, for example, when it is desired to inhibit the cell cytotoxicity induced by TNF it would be desired to enhance NF-κB induction by enhancing the TRAF2-NIK interaction or by enhancing TRAF2- and/or NIK- specific NF-κB induction. There are many diseases in which such drugs can be of great help. Amongst others, (see above discussion as well) acute hepatitis in which the acute damage to the liver seems to reflect FAS/APO1 receptor-mediated death of the liver cells following induction by the Fas ligand; autoimmune-induced cell death such as the death of the β Langerhans cells of the pancreas, that results in diabetes; the death of cells in graft rejection (e.g., kidney, heart and liver); the death of oligodendrocytes in the brain in multiple sclerosis; and AIDS-inhibited T cell suicide which causes proliferation of the AIDS virus and hence the AIDS disease.

In such cases, it would be desired to inhibit the FAS/APO1 receptor-mediated cell cytotoxicity (apoptosis) pathway and enhance the FAS/APO1 receptor-mediated induction of NF-κB via TRAF2 and the TRAF2-NIK interaction. One way of doing this would be to increase the amount of NIK in the cells or to increase the amount of TRAF2 and NIK so that the NIK- or TRAF2-NIK- mediated induction of NF-κB activation will be increased providing higher levels of NF-κB activation and hence cell survival; or so that the direct or indirect interaction between FAS/APO1 receptor and TRAF2 (or TRAF2-NIK) will be increased resulting in a decrease in FAS/APO1 receptor interactions with cell cytotoxic mediators (e.g. MACH, see scheme in Fig. 2b) to provide for an increase in the induction of NF-κB activation and cell survival.

Conversely, in the case of, for example, tumors and infected cells (see also discussion above) it would be desired to increase the FAS/APO1 receptor-mediated cell cytotoxicity to bring about increased cell death. In this case it would be desired to inhibit FAS/APO1 receptor-TRAF2 (or -TRAF2-NIK) interactions and/or to inhibit NIK directly, and thereby to decrease the induction of NF-κB activity.

It is possible that NIK or one or more of its possible isoforms, analogs or fragments may serve as "natural" inhibitors of NIK itself or of the NIK-TRAF2 interaction, and as such serve as inhibitors of the induction of NF- κ B activation. Such inhibitors may thus be employed as the specific inhibitors noted above, for example, those inhibitors to be used when it is desired to increase the cell cytotoxic effects of TNF or the ligand of the FAS/APO1 receptor in order to increase cell death. In fact, as exemplified herein below, various NIK analogs and muteins have been isolated in accordance with the present invention, which are kinase-deficient analogs/muteins and which are capable of blocking the induction of NF- κ B activation mediated by the TNF receptors, the FAS/APO1 receptor, their associated proteins TRADD, RIP and MORT1; as well as mediated by the IL-1 receptor (which activation is via NIK but independent of TRAF2); and also as mediated by bacterial endotoxin (LPS), forbol myristate acetate, and the HTLV-1 protein TAX. Likewise, other substances such as peptides, organic compounds, antibodies, etc. may also be screened to obtain specific drugs which are capable of inhibiting the TRAF2-NIK interaction or the activity of NIK.

In a similar fashion, when it is desired to increase the NF- κ B activation in various situations as noted above it is possible, for example, to increase the amount of NIK and/or TRAF2 in cells by various standard methods noted herein above (e.g. introducing DNA encoding NIK and/or TRAF2 into cells to induce increased expression, or preparing suitable formulations containing NIK and/or TRAF2 for direct introduction into cells, or any other way known to those of skill in the art). Likewise, other substances such as peptides, organic compounds, etc. may also be screened to obtain specific drugs which are capable of enhancing the activity of NIK or of enhancing the TRAF2-NIK interaction.

A non-limiting example of how peptide inhibitors of the NIK-TRAF2 interaction would be designed and screened is based on previous studies on peptide inhibitors of ICE or ICE-like proteases, the substrate specificity of ICE and strategies for epitope analysis using peptide synthesis. The minimum requirement for efficient cleavage of a peptide by ICE was found to involve four amino acids to the left of the cleavage site with a strong preference for aspartic acid in the P₁ position and with methylamine being sufficient to the right of the P₁ position (Sleath et al., 1990; Howard et al., 1991; Thornberry et al., 1992). Furthermore, the fluorogenic substrate peptide (a tetrapeptide), acetyl-Asp-Glu-Val-Asp-a (4-methyl-coumaryl-7-amide) abbreviated Ac-DEVD-AMC, corresponds to a sequence in

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poly (ADP-ribose) polymerase (PARP) found to be cleaved in cells shortly after FAS-R stimulation, as well as other apoptotic processes (Kaufmann, 1989; Kaufmann et al., 1993; Lazebnik et al., 1994), and is cleaved effectively by CPP32 (a member of the CED3/ICE protease family) and MACH proteases.

As Asp in the P₁ position of the substrate appears to be important, tetrapeptides having Asp as the fourth amino acid residue and various combinations of amino acids in the first three residue positions can be rapidly screened for binding to the active site of the proteases using, for example, the method developed by Geysen (Geysen, 1985; Geysen et al., 1987) where a large number of peptides on solid supports were screened for specific interactions with antibodies. The binding of MACH proteases to specific peptides can be detected by a variety of well known detection methods within the skill of those in the art, such as radiolabeling, etc. This method of Geysen's was shown to be capable of testing at least 4000 peptides each working day.

In a similar way the exact binding region or region of homology which determines the interaction between TRAF2 and NIK (or any other TRAF protein and TRAF-binding protein) can be elucidated and then peptides may be screened which can serve to block this interaction, e.g. peptides synthesized having a sequence similar to that of the binding region or complementary thereto which can compete with natural NIK (or TRAF-binding protein) for binding to TRAF2 (or TRAF).

Since it may be advantageous to design peptide inhibitors that selectively inhibit TRAF2-NIK (or TRAF-TRAF binding protein) interactions without interfering with physiological cell death processes in which other members of the intracellular signaling pathway are involved, e.g. MACH proteases of the cell death pathway, which are members of the CED3/ICE family of proteases, the pool of peptides binding to TRAF2 (or TRAF) or NIK (or TRAF-binding proteins) in an assay such as the one described above can be further synthesized as a fluorogenic substrate peptide to test for selective binding to such other proteins to select only those specific for TRAF2/NIK (or TRAF/TRAF-binding protein). Peptides which are determined to be specific for, for example, TRAF2/NIK, can then be modified to enhance cell permeability and inhibit the activity of TRAF2 and/or NIK either reversibly or irreversibly. Thornberry et al. (1994) reported that a tetrapeptide (acyloxy methyl ketone Ac-Tyr-Val-Ala-Asp-CH₂OC (O)-[2,6-(CF₃)₂] Ph was a potent inactivator of ICE. Similarly, Milligan et al. (1995) reported that tetrapeptide inhibitors having a

chloromethylketone (irreversibly) or aldehyde (reversibly) groups inhibited ICE.³⁸
In addition, a benzyloxycarbonyl-Asp-CH₂OC (O) -2,6-dichlorobenzene (DCB) was shown
to inhibit ICE (Mashima et al., 1995). Accordingly, in an analogous way, tetrapeptides that
selectively bind to, for example, TRAF2 or NIK, can be modified with, for example, an
aldehyde group, chloromethylketone, (acyloxy) methyl ketone or a CH₂OC (O)-DCB group
to create a peptide inhibitor of TRAF2/NIK activity. Further, to improve permeability,
peptides can be, for example, chemically modified or derivatized to enhance their
permeability across the cell membrane and facilitate the transport of such peptides through
the membrane and into the cytoplasm. Muranishi et al. (1991) reported derivatizing
thyrotropin-releasing hormone with lauric acid to form a lipophilic lauroyl derivative with
good penetration characteristics across cell membranes. Zacharia et al. (1991) also reported
the oxidation of methionine to sulfoxide and the replacement of the peptide bond with its
ketomethylene isoester (COCH₂) to facilitate transport of peptides through the cell
membrane. These are just some of the known modifications and derivatives that are well
within the skill of those in the art.

Furthermore, drug or peptide inhibitors, which are capable of inhibiting the activity
of, for example, NIK by inhibiting the NIK-TRAF2 interaction and likewise, the interaction
between TRAF proteins and TRAF-binding proteins can be conjugated or complexed with
molecules that facilitate entry into the cell.

U.S. Patent 5,149,782 discloses conjugating a molecule to be transported across the
cell membrane with a membrane blending agent such as fusogenic polypeptides, ion-channel
forming polypeptides, other membrane polypeptides, and long chain fatty acids, e.g.
myristic acid, palmitic acid. These membrane blending agents insert the molecular
conjugates into the lipid bilayer of cellular membranes and facilitate their entry into the
cytoplasm.

Low et al., U.S. Patent 5,108,921, reviews available methods for transmembrane
delivery of molecules such as, but not limited to, proteins and nucleic acids by the
mechanism of receptor mediated endocytotic activity. These receptor systems include those
recognizing galactose, mannose, mannose 6-phosphate, transferrin, asialoglycoprotein,
transcobalamin (vitamin B₁₂), α -2 macroglobulins, insulin and other peptide growth factors
such as epidermal growth factor (EGF). Low et al. teaches that nutrient receptors, such as
receptors for biotin and folate, can be advantageously used to enhance transport across the

cell membrane due to the location and multiplicity of biotin and folate receptors on the membrane surfaces of most cells and the associated receptor mediated transmembrane transport processes. Thus, a complex formed between a compound to be delivered into the cytoplasm and a ligand, such as biotin or folate, is contacted with a cell membrane bearing biotin or folate receptors to initiate the receptor mediated trans-membrane transport mechanism and thereby permit entry of the desired compound into the cell.

ICE is known to have the ability to tolerate liberal substitutions in the P₂ position and this tolerance to liberal substitutions was exploited to develop a potent and highly selective affinity label containing a biotin tag (Thornberry et al., 1994). Consequently, the P₂ position as well as possibly the N-terminus of the tetrapeptide inhibitor can be modified or derivatized, such as to with the addition of a biotin molecule, to enhance the permeability of these peptide inhibitors across the cell membrane.

In addition, it is known in the art that fusing a desired peptide sequence with a leader/signal peptide sequence to create a "chimeric peptide" will enable such a "chimeric peptide" to be transported across the cell membrane into the cytoplasm.

As will be appreciated by those of skill in the art of peptides, the peptide inhibitors of the TRAF-TRAF-binding protein interaction, for example, the TRAF2-NIK interaction according to the present invention is meant to include peptidomimetic drugs or inhibitors, which can also be rapidly screened for binding to, for example TRAF2/NIK to design perhaps more stable inhibitors.

It will also be appreciated that the same means for facilitating or enhancing the transport of peptide inhibitors across cell membranes as discussed above are also applicable to the TRAF-binding proteins, for example, NIK, its analogs, fragments or its isoforms themselves as well as other peptides and proteins which exert their effects intracellularly.

As regards the antibodies mentioned herein throughout, the term "antibody" is meant to include polyclonal antibodies, monoclonal antibodies (mAbs), chimeric antibodies, anti-idiotypic (anti-Id) antibodies to antibodies that can be labeled in soluble or bound form, as well as fragments thereof provided by any known technique, such as, but not limited to enzymatic cleavage, peptide synthesis or recombinant techniques.

Polyclonal antibodies are heterogeneous populations of antibody molecules derived from the sera of animals immunized with an antigen. A monoclonal antibody contains a substantially homogeneous population of antibodies specific to antigens, which populations

contains substantially similar epitope binding sites. MABs may be obtained by methods known to those skilled in the art. See, for example Kohler and Milstein, Nature, 256:495-497 (1975); U.S. Patent No. 4,376,110; Ausubel et al., eds., Harlow and Lane ANTIBODIES: A LABORATORY MANUAL, Cold Spring Harbor Laboratory (1988);
5 and Colligan et al., eds., Current Protocols in Immunology, Greene Publishing Assoc. and Wiley Interscience N.Y., (1992-1996), the contents of which references are incorporated entirely herein by reference. Such antibodies may be of any immunoglobulin class including IgG, IgM, IgE, IgA, GILD and any subclass thereof. A hybridoma producing a mAb of the present invention may be cultivated *in vitro*, *in situ* or *in vivo*. Production of high titers of
10 mAbs *in vivo* or *in situ* makes this the presently preferred method of production.

Chimeric antibodies are molecules of which different portions are derived from different animal species, such as those having the variable region derived from a murine mAb and a human immunoglobulin constant region. Chimeric antibodies are primarily used to reduce immunogenicity in application and to increase yields in production, for example,
15 where murine mAbs have higher yields from hybridomas but higher immunogenicity in humans, such that human/murine chimeric mAbs are used. Chimeric antibodies and methods for their production are known in the art (Cabilly et al., *Proc. Natl. Acad. Sci. USA* 81:3273-3277 (1984); Morrison et al., *Proc. Natl. Acad. Sci. USA* 81:6851-6855 (1984); Boulianne et al., *Nature* 312:643-646 (1984); Cabilly et al., European Patent
20 Application 125023 (published November 14, 1984); Neuberger et al., *Nature* 314:268-270 (1985); Taniguchi et al., European Patent Application 171496 (published February 19, 1985); Morrison et al., European Patent Application 173494 (published March 5, 1986); Neuberger et al., PCT Application WO 8601533, (published March 13, 1986); Kudo et al., European Patent Application 184187 (published June 11, 1986); Sahagan et al., *J.*
25 *Immunol.* 137:1066-1074 (1986); Robinson et al., International Patent Application No. WO8702671 (published May 7, 1987); Liu et al., *Proc. Natl. Acad. Sci. USA* 84:3439-3443 (1987); Sun et al., *Proc. Natl. Acad. Sci. USA* 84:214-218 (1987); Better et al., *Science* 240:1041-1043 (1988); and Harlow and Lane, ANTIBODIES: A LABORATORY MANUAL, supra. These references are entirely incorporated herein by reference.

30 An anti-idiotypic (anti-Id) antibody is an antibody which recognizes unique determinants generally associated with the antigen-binding site of an antibody. An Id antibody can be prepared by immunizing an animal of the same species and genetic type

(e.g. mouse strain) as the source of the mAb to which an anti-Id is being prepared. The immunized animal will recognize and respond to the idiotypic determinants of the immunizing antibody by producing an antibody to these idiotypic determinants (the anti-Id antibody). See, for example, U.S. Patent No. 4,699,880, which is herein entirely
5 incorporated by reference.

The anti-Id antibody may also be used as an "immunogen" to induce an immune response in yet another animal, producing a so-called anti-anti-Id antibody. The anti-anti-Id may be epitopically identical to the original mAb which induced the anti-Id. Thus, by using antibodies to the idiotypic determinants of a mAb, it is possible to identify other
10 clones expressing antibodies of identical specificity.

Accordingly, mAbs generated against the TRAF-binding proteins, analogs, fragments or derivatives thereof, (e.g. NIK, its isoforms, analogs, fragments or derivatives) of the present invention may be used to induce anti-Id antibodies in suitable animals, such as BALB/c mice. Spleen cells from such immunized mice are used to produce anti-Id
15 hybridomas secreting anti-Id mAbs. Further, the anti-Id mAbs can be coupled to a carrier such as keyhole limpet hemocyanin (KLH) and used to immunize additional BALB/c mice. Sera from these mice will contain anti-anti-Id antibodies that have the binding properties of the original mAb specific for an epitope of the above TRAF-binding protein, or analogs, fragments and derivatives thereof.

The anti-Id mAbs thus have their own idiotypic epitopes, or "idiotopes" structurally similar to the epitope being evaluated, such as GRB protein-a.

The term "antibody" is also meant to include both intact molecules as well as fragments thereof, such as, for example, Fab and F(ab')₂, which are capable of binding antigen. Fab and F(ab')₂ fragments lack the Fc fragment of intact antibody, clear more
25 rapidly from the circulation, and may have less non-specific tissue binding than an intact antibody (Wahl et al., *J. Nucl. Med.* 24:316-325 (1983)).

It will be appreciated that Fab and F(ab')₂ and other fragments of the antibodies useful in the present invention may be used for the detection and quantitation of the TRAF-binding protein according to the methods disclosed herein for intact antibody molecules.

30 Such fragments are typically produced by proteolytic cleavage, using enzymes such as papain (to produce Fab fragments) or pepsin (to produce F(ab')₂ fragments).

An antibody is said to be "capable of binding" a molecule if it is capable of specifically reacting with the molecule to thereby bind the molecule to the antibody. The term "epitope" is meant to refer to that portion of any molecule capable of being bound by an antibody which can also be recognized by that antibody. Epitopes or "antigenic determinants" usually consist of chemically active surface groupings of molecules such as amino acids or sugar side chains and have specific three dimensional structural characteristics as well as specific charge characteristics.

An "antigen" is a molecule or a portion of a molecule capable of being bound by an antibody which is additionally capable of inducing an animal to produce antibody capable of binding to an epitope of that antigen. An antigen may have one or more than one epitope. The specific reaction referred to above is meant to indicate that the antigen will react, in a highly selective manner, with its corresponding antibody and not with the multitude of other antibodies which may be evoked by other antigens.

The antibodies, including fragments of antibodies, useful in the present invention may be used to quantitatively or qualitatively detect the TRAF-binding protein (e.g. NIK) in a sample or to detect presence of cells which express the TRAF-binding protein of the present invention. This can be accomplished by immunofluorescence techniques employing a fluorescently labeled antibody (see below) coupled with light microscopic, flow cytometric, or fluorometric detection.

The antibodies (or fragments thereof) useful in the present invention may be employed histologically, as in immunofluorescence or immunoelectron microscopy, for *in situ* detection of the TRAF-binding protein of the present invention. *In situ* detection may be accomplished by removing a histological specimen from a patient, and providing the labeled antibody of the present invention to such a specimen. The antibody (or fragment) is preferably provided by applying or by overlaying the labeled antibody (or fragment) to a biological sample. Through the use of such a procedure, it is possible to determine not only the presence of the TRAF-binding protein, but also its distribution on the examined tissue. Using the present invention, those of ordinary skill will readily perceive that any of wide variety of histological methods (such as staining procedures) can be modified in order to achieve such *in situ* detection.

Such assays for the TRAF-binding protein of the present invention typically comprises incubating a biological sample, such as a biological fluid, a tissue extract, freshly

harvested cells such as lymphocytes or leukocytes, or cells which have been incubated in tissue culture, in the presence of a detectably labeled antibody capable of identifying the TRAF-binding protein, and detecting the antibody by any of a number of techniques well known in the art.

5 The biological sample may be treated with a solid phase support or carrier such as nitrocellulose, or other solid support or carrier which is capable of immobilizing cells, cell particles or soluble proteins. The support or carrier may then be washed with suitable buffers followed by treatment with a detectably labeled antibody in accordance with the present invention, as noted above. The solid phase support or carrier may then be washed
10 with the buffer a second time to remove unbound antibody. The amount of bound label on said solid support or carrier may then be detected by conventional means.

By "solid phase support", "solid phase carrier", "solid support", "solid carrier", "support" or "carrier" is intended any support or carrier capable of binding antigen or antibodies. Well-known supports or carriers, include glass, polystyrene, polypropylene,
15 polyethylene, dextran, nylon amylases, natural and modified celluloses, polyacrylamides, gabbros and magnetite. The nature of the carrier can be either soluble to some extent or insoluble for the purposes of the present invention. The support material may have virtually any possible structural configuration so long as the coupled molecule is capable of binding to an antigen or antibody. Thus, the support or carrier configuration may be
20 spherical, as in a bead, cylindrical, as in the inside surface of a test tube, or the external surface of a rod. Alternatively, the surface may be flat such as a sheet, test strip, etc. Preferred supports or carriers include polystyrene beads. Those skilled in the art will know may other suitable carriers for binding antibody or antigen, or will be able to ascertain the same by use of routine experimentation.

25 The binding activity of a given lot of antibody, of the invention as noted above, may be determined according to well known methods. Those skilled in the art will be able to determine operative and optimal assay conditions for each determination by employing routine experimentation.

30 Other such steps as washing, stirring, shaking, filtering and the like may be added to the assays as is customary or necessary for the particular situation.

One of the ways in which an antibody in accordance with the present invention can be detectably labeled is by linking the same to an enzyme and used in an enzyme

immunoassay (EIA). This enzyme, in turn, when later exposed to an appropriate substrate, will react with the substrate in such a manner as to produce a chemical moiety which can be detected, for example, by spectrophotometric, fluorometric or by visual means. Enzymes which can be used to detectably label the antibody include, but are not limited to, malate dehydrogenase, staphylococcal nuclease, delta-5-steroid isomerase, yeast alcohol dehydrogenase, alpha-glycerophosphate dehydrogenase, triose phosphate isomerase, horseradish peroxidase, alkaline phosphatase, asparaginase, glucose oxidase, beta-galactosidase, ribonuclease, urease, catalase, glucose-6-phosphate dehydrogenase, glucoamylase and acetylcholin-esterase. The detection can be accomplished by colorimetric methods which employ a chromogenic substrate for the enzyme. Detection may also be accomplished by visual comparison of the extent of enzymatic reaction of a substrate in comparison with similarly prepared standards.

Detection may be accomplished using any of a variety of other immunoassays. For example, by radioactive labeling the antibodies or antibody fragments, it is possible to detect R-PTPase through the use of a radioimmunoassay (RIA). A good description of RIA may be found in Laboratory Techniques and Biochemistry in Molecular Biology, by Work, T.S. et al., North Holland Publishing Company, NY (1978) with particular reference to the chapter entitled "An Introduction to Radioimmune Assay and Related Techniques" by Chard, T., incorporated by reference herein. The radioactive isotope can be detected by such means as the use of a g counter or a scintillation counter or by autoradiography.

It is also possible to label an antibody in accordance with the present invention with a fluorescent compound. When the fluorescently labeled antibody is exposed to light of the proper wavelength, its presence can be then detected due to fluorescence. Among the most commonly used fluorescent labeling compounds are fluorescein isothiocyanate, rhodamine, phycoerythrin, phycocyanin, allophycocyanin, o-phthaldehyde and fluorescamine.

The antibody can also be detectably labeled using fluorescence emitting metals such as ^{152}E , or others of the lanthanide series. These metals can be attached to the antibody using such metal chelating groups as diethylenetriamine pentaacetic acid (ETPA).

The antibody can also be detectably labeled by coupling it to a chemiluminescent compound. The presence of the chemiluminescent-tagged antibody is then determined by detecting the presence of luminescence that arises during the course of a chemical reaction.

Examples of particularly useful chemiluminescent labeling compounds are luminol, isoluminol, therrromatic acridinium ester, imidazole, acridinium salt and oxalate ester.

Likewise, a bioluminescent compound may be used to label the antibody of the present invention. Bioluminescence is a type of chemiluminescence found in biological systems in which a catalytic protein increases the efficiency of the chemiluminescent reaction. The presence of a bioluminescent protein is determined by detecting the presence of luminescence. Important bioluminescent compounds for purposes of labeling are luciferin, luciferase and aequorin.

An antibody molecule of the present invention may be adapted for utilization in an immunometric assay, also known as a "two-site" or "sandwich" assay. In a typical immunometric assay, a quantity of unlabeled antibody (or fragment of antibody) is bound to a solid support or carrier and a quantity of detectably labeled soluble antibody is added to permit detection and/or quantitation of the ternary complex formed between solid-phase antibody, antigen, and labeled antibody.

Typical, and preferred, immunometric assays include "forward" assays in which the antibody bound to the solid phase is first contacted with the sample being tested to extract the antigen from the sample by formation of a binary solid phase antibody-antigen complex. After a suitable incubation period, the solid support or carrier is washed to remove the residue of the fluid sample, including unreacted antigen, if any, and then contacted with the solution containing an unknown quantity of labeled antibody (which functions as a "reporter molecule"). After a second incubation period to permit the labeled antibody to complex with the antigen bound to the solid support or carrier through the unlabeled antibody, the solid support or carrier is washed a second time to remove the unreacted labeled antibody.

In another type of "sandwich" assay, which may also be useful with the antigens of the present invention, the so-called "simultaneous" and "reverse" assays are used. A simultaneous assay involves a single incubation step as the antibody bound to the solid support or carrier and labeled antibody are both added to the sample being tested at the same time. After the incubation is completed, the solid support or carrier is washed to remove the residue of fluid sample and uncomplexed labeled antibody. The presence of

labeled antibody associated with the solid support or carrier is then determined as it would be in a conventional "forward" sandwich assay.

In the "reverse" assay, stepwise addition first of a solution of labeled antibody to the fluid sample followed by the addition of unlabeled antibody bound to a solid support or carrier after a suitable incubation period is utilized. After a second incubation, the solid phase is washed in conventional fashion to free it of the residue of the sample being tested and the solution of unreacted labeled antibody. The determination of labeled antibody associated with a solid support or carrier is then determined as in the "simultaneous" and "forward" assays.

As mentioned above, the present invention also relates to pharmaceutical compositions comprising recombinant animal virus vectors encoding the TRAF-binding proteins, which vector also encodes a virus surface protein capable of binding specific target cell (e.g., cancer cells) surface proteins to direct the insertion of the TRAF-binding protein sequences into the cells. Further pharmaceutical compositions of the invention comprises as the active ingredient (a) an oligonucleotide sequence encoding an anti-sense sequence of the TRAF-binding protein sequence, or (b) drugs that block the TRAF-binding protein- TRAF interaction.

Pharmaceutical compositions according to the present invention include a sufficient amount of the active ingredient to achieve its intended purpose. In addition, the pharmaceutical compositions may contain suitable pharmaceutically acceptable carriers comprising excipients and auxiliaries which facilitate processing of the active compounds into preparations which can be used pharmaceutically and which can stabilize such preparations for administration to the subject in need thereof as are well known to those of skill in the art.

The TRAF-binding protein and its isoforms or isotypes are suspected to be expressed in different tissues at markedly different levels and apparently also with different patterns of isotypes in an analogous fashion to the expression of various other proteins involved in the intracellular signaling pathways as indicated in the above listed co-owned co-pending patent applications. These differences may possibly contribute to the tissue-specific features of response to the Fas/APO1-ligand and TNF. As in the case of other CED3/ICE homologs (Wang et al., 1994; Alnemri et al., 1995), the present inventors have previously shown (in the above mentioned patent applications) that MACH isoforms that

contain incomplete CED3/ICE regions (e.g., MACH α 3) are found to have an inhibitory effect on the activity of co-expressed MACH α 1 or MACH α 2 molecules; they are also found to block death induction by Fas/APO1 and p55-R. Expression of such inhibitory isoforms in cells may constitute a mechanism of cellular self-protection against Fas/APO1- and TNF-mediated cytotoxicity. The wide heterogeneity of MACH isoforms, which greatly exceeds that observed for any of the other proteases of the CED3/ICE family, should allow a particularly fine tuning of the function of the active MACH isoforms.

In accordance with the present invention there have also been isolated analogs/mutins of one of the TRAF-binding proteins, namely of the TRAF2-binding protein NIK. These NIK analogs/mutins (see above and see Examples below) are inhibitory to NIK-mediated as well as inhibitory to the induction of NF- κ B activation mediated by the TNF receptors, FAS/APO1 receptor, their related proteins, the IL-1 receptor and other agents. Hence, as noted above, the TRAF-binding proteins or possible isoforms may have varying effects in different tissues as regards their interaction with TRAF proteins and their influence thereby on the activity of the TRAF proteins, or intracellular signaling mediated by the TRAF proteins.

It is also possible that some of the possible TRAF-binding protein isoforms serve other functions. For example, NIK or some NIK analogs, or isoforms may also act as docking sites for molecules that are involved in other, non-cytotoxic effects of, for example, Fas/APO1 and TNF receptors via interaction with TRAF2 or even independently of TRAF2.

Due to the unique ability of Fas/APO1 and TNF receptors to cause cell death, as well as the ability of the TNF receptors to trigger other tissue-damaging activities, aberrations in the function of these receptors could be particularly deleterious to the organism. Indeed, both excessive and deficient functioning of these receptors have been shown to contribute to pathological manifestations of various diseases (Vassalli, 1992; Nagata and Golstein, 1995). Identifying the molecules that participate in the signaling activity of the receptors, and finding ways to modulate the activity of these molecules, could direct new therapeutic approaches. In view of the suspected important role of TRAF proteins, e.g. TRAF2 and hence the TRAF-TRAF-binding protein, e.g. TRAF2-NIK interaction in Fas/APO1- and TNF-mediated NF- κ B activation, it seems particularly important to design drugs that can block the TRAF-TRAF binding protein interaction, e.g.

TRAF2-NIK interaction when it is desired to kill cells (by inhibiting NF- κ B activation), and conversely, when it is desired to preserve cells this interaction should be enhanced (to enhance NF- κ B activation).

The present invention also concerns proteins or other ligands which can bind to the TRAF-binding proteins of the invention and thereby modulate/mediate the activity of the TRAF-binding proteins. Such proteins or ligands may be screened, isolated and produced by any of the above mentioned methods. For example, there may be isolated a number of new ligands, including proteins, capable of binding to the NIK proteins of the invention (such new proteins/ligands excluding the known TRAF2 and possibly I κ B if NIK actually binds I- κ B).

As detailed above, such new TRAF-binding protein-binding proteins/ligands, e.g. NIK-binding proteins, may serve as, for example, inhibitors or enhancers of NIK-mediated activity or the activity mediated by the, for example, TRAF2-NIK interaction, and as such will have important roles in various pathological and other situations as detailed above. Another function of such TRAF-binding protein-binding proteins/ligands would be to serve as specific agents for the purification of the TRAF-binding proteins by, for example, affinity chromatography, these new binding proteins/ligands being attached to the suitable chromatography matrices to form the solid or affinity support/matrix through which a solution, extract or the like, containing the TRAF-binding proteins, e.g. NIK, will be passed and in this way to facilitate the purification thereof. Such methods of affinity chromatography are now well known and generally standard procedures of the art.

Likewise, all of the above mentioned TRAF-binding proteins, analogs, fragments, isoforms and derivatives of the present invention may be used to purify by affinity chromatography the various TRAF proteins to which they bind. For example, TRAF2-binding proteins like NIK, and analogs, fragments and muteins of NIK (see examples below) may be used for the affinity chromatography purification of TRAF2. Hence in the same way as the NIK protein, analogs/muteins of the present invention were isolated and produced (see Examples below) using these methods and any other equivalent methods readily apparent to those of skill in the art (as detailed herein above), any other TRAF2-binding proteins may be identified and produced. Such a method for identifying and producing these TRAF-binding proteins, e.g. TRAF2-binding proteins will include a screening step in which the TRAF (e.g. TRAF2) protein, or at least a specific portion

thereof (e.g. the portion of TRAF2 between a.a. 222-501) is used as a substrate or 'bait' to obtain proteins or any other ligand capable of binding thereto; followed by steps of identifying and characterizing such proteins/ligands so-obtained; and subsequently producing such proteins/ligands in substantially isolated and purified forms. All these steps are well known to those of skill in the art and are detailed herein above and herein below.

The invention will now be described in more detail in the following non-limiting examples and the accompanying drawings :

It should also be noted that the procedures of :

- i) two-hybrid screen and two-hybrid β -galactosidase expression test; (ii) induced expression, metabolic labeling and immunoprecipitation of proteins; (iii) *in vitro* binding; (iv) assessment of the cytotoxicity; and (v) Northern and sequence analyses, as well as other procedures used in the following Examples have been detailed in previous publications by the present inventors in respect of other intracellular signaling proteins and pathways (see, for example, Boldin et al., 1995a, 1995b, and Boldin et al. 1996). These procedures also appear in detail in the co-owned co-pending Israel Application Nos. 114615, 114986, 115319, 116588, 117932, and 120367 as well as the corresponding PCT application No. PCT/US96/10521). Accordingly, the full disclosures of all these publications and patent applications are included herein in their entirety and at least as far as the detailed experimental procedures are concerned.

EXAMPLES

Materials and Methods

i) cDNA libraries

a) B-cell cDNA library

Oligo dT primed library constructed from human B cells was used (Durfee et al., 1993). The cDNAs of the library were inserted into the XhoI site of the pACT based vector pSE1107 in fusion with GAL4 activation domain.

b) λ gt10 testis cDNA library

A cDNA library from human testis was used. The library is a random hexanucleotide primed library with an average insert size of 200 to 400 bp.

ii) Yeast strains

Two yeast strains were used as hoststrains for transformation and screening: HF7c strain that was used in the two hybrid screen and SFY526 strain that was used in the β -galactosidase assays. Both strains carry the auxotrophic markers *trp1* and *leu2*, namely these yeast strains cannot grow in minimal synthetic medium lacking tryptophan and leucine, unless they are transformed by a plasmid carrying the wild-type versions of these genes (*TRP1*, *LEU2*). The two yeast strains carry deletion mutations in their *GAL4* and *GAL80* genes (*gal4-542* and *gal80-538* mutations, respectively).

SFY526 and HF7c strains carry the *lacZ* reporter in their genotypes; in SFY526 strain fused to the UAS and the TATA portion of *GAL1* promoter, and in HF7c three copies of the *GAL4* 17-mer consensus sequence and the TATA portion of the *CYC1* promoter are fused to *lacZ*. Both *GAL1* UAS and the *GAL4* 17-mers are responsive to the *GAL4* transcriptional activator. In addition, HF7c strain carries the *HIS3* reporter fused to the UAS and the TATA portion of *GAL1* promoter.

iii) Cloning of human TRAF2

The human TRAF2 was cloned by PCR from an HL60 cDNA library (for TRAF2 sequence and other details see Rothe et al., 1994; Rothe et al., 1995a; Cheng et al., 1996; Hsu et al., 1996; and Wallach, 1996). The primers used were: a) 30-mer forward primer CAGGATCCTCATGGCTGCAGCTAGCGTGAC corresponding to the coding sequence of hTRAF2 starting from the codon for the first methaonine (underlined) and including a linker with BamHI site. b) 32-mer reverse primer GGTCGACTTAGAGCCCTGTCAGGTCCACAATG that includes hTRAF2 gene stop codon (underlined) and a SalI restriction site in its linker. PCR program comprised of an initial denaturation step 2 min. at 94°C followed by 30 cycles of 1 min. at 94°C, 1 min. at 64°C, 1 min. and 40 sec. at 72°C. The amplified human TRAF2 was then inserted into the BamHI - SalI sites of pGBT9 vector in conjunction with GAL 4 DNA Binding domain.

iv) Two hybrid screen of B-cell library

The two hybrid screen is a technique (see details in above mentioned publications and patent applications) used in order to identify factors that are associated with a particular molecule that serves as a "bait". In the present invention TRAF2 that was cloned into the vector pGBT9, served as the bait. TRAF2 was co-expressed together with the screened B-cell cDNA library in the yeast strain HF7c. The PCR-cloned TRAF2 was a

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recombinant fusion with the CAL4 DNA- binding domain and the screened cDNA library was fused to the GAL4 activation domain in the pSE1107 vector. The reporter gene in HF7c was HIS3 fused to the upstream activating sequence (UAS) of the GAL1 promoter which is responsive to GAL4 transcriptional activator. Transformants that contained both pGBT9 and pSE1107 plasmids were selected for growth on plates without tryptophan and leucine. In a second step positive clones which expressed two hybrid proteins that interact with each other, and therefore activated GAL1-HIS3, were picked up from plates devoided of tryptophan, leucine and histidine and contained 50 mM 3-aminotriazol (3AT).

v) β -galactosidase assay

Positive clones picked up in the two hybriide screen were subjected to lacZ color development test in SFY526 yeast cells, following Clontech Laboratories' manual (for details see above mentioned publications and patent applications). In brief, transformants were allowed to grow at 30°C for 2-4 days until reaching about 2 mm in diameter, then were transferred onto Whatman filters. The filters went through a freeze/thaw treatment in order to permeabilize the cells, then soaked in a buffer (16.1 mg/ml $\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$; 5.5 mg/ml $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$; 0.75 mg/ml KCl; 0.75 mg/ml $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, pH=7) containing 0.33 mg/ml X-gal and 0.35 mM β -mercaptoethanol. Colonies were monitored for development of blue color which is an indication for induction of β -galactosidase.

vi) Expression of cloned cDNAs

Two kinds of expression vectors were constructed:

- a) A pUHD10-3 based vectors containing the open reading frame (ORF) of either clone 9, 10 or 15 in fusion with the Hemeaglutinine (HA) epitope.
- b) A pUHD10-3 based vector into which FLAG octapeptide sequence was introduced just in front of cloned TRAF2, hereby named FLAG/B6/TRAF2.

The constructs containing an ORF of clone 9, 10 or 15 were transfected into HeLa-Bujard cells (for these cells see Gossen, M. and Bujard, M. (1992)) either alone or cotransfected with FLAG/B6/TRAF2 using standard calcium-phosphate method (Method in, for example, Current Protocols in Molecular Biology, eds. Ausubel, F.M et al.)

vii) Luciferase assay

Typically 5×10^5 transfected cells were harvested by washing three times with cold PBS and resuspending in 400 μl extraction buffer (0.1 M $\text{K}_2\text{HPO}_4/\text{KH}_2\text{PO}_4$ pH=7.8; 1 mM

DTT). Lysis of the cells was achieved by three times freezing in liquid nitrogen and thawing. Cell debris was removed by centrifugation (5 min. at 10,000 x g). For the luciferase assay, 200 μ l of luciferase buffer (25 mM glycylglycine, 15 mM K_2HPO_4/KH_2PO_4 pH=7.8, 15 mM $MgSO_4$, 4 mM EGTA, 2 mM ATP, 1 mM DTT) were added to 50 μ l of the lysate. Subsequently, 100 μ l of 0.2 mM D-luciferine, 25 mM glycylglycine, 1 mM DTT were added to the reaction. Luciferase activity was determined by reading light emission using a Lumitron luminometer set on 10 seconds integration (see above publications and patent applications for additional details).

Example 1: Cloning of new clones 9, 10 and 15

A cDNA library prepared from B-cells was screened for proteins that associate with TRAF2, using the two hybrid technique as described in Materials and Methods (iv). Only in transformants that expressed both TRAF2 and a protein capable of interacting with it, the GAL4 DNA-binding domain and the transcriptional activation domain were brought together. The result was the activation and expression of the reporter gene, in this case HIS3 fused to the UAS and the TATA portion of the GAL1 promoter.

The screen yielded approximately 2000 clones which were able to grow on Trp-, Leu-, His- 3AT plates. DNA prepared from 165 randomly selected positive clones served for transient co-transfection of SFY526 yeast strain together with TRAF2 cloned into pGBT9 vector. Assay for β -galactosidase activity was performed on the transformed SFY526 yeast colonies as described in Materials and Methods (v). The blue color that developed was an indication for yeast colonies that contain cDNA encoding a protein or polypeptide that binds to TRAF2.

The results of the two hybide screen; the ability of the picked clones to grow on 3AT plates and to induce LacZ as measured in the color test, are summarized in Table 1. Of the positive clones checked, two were cDNAs coding for known proteins; TRAF2 itself that is capable of self-associating and forming homodimers, and the lymphotoxin beta receptor whose intacellular domains were shown to bind TRAF2. Three of the cloned cDNAs (clones 9, 10 and 15) were novel.

The positive clones were further checked in a binding specificity test, namely checked for their interaction with irrelevant baits. As shown in Table 2, clones 9 and 10 reacted only with TRAF2 and did not bind to any one of a number of irrelevant proteins

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checked. Clone 15, on the other hand, did not bind to MORT1, nor to the intercellular domains of the p55 and p75 TNF receptors, but did weakly bind to Lamin and to Cycline D.

In order to narrow down the region on TRAF2 molecule which interact with clones 9, 10 and 15, two additional constructs were made. One construct comprised of the N-terminal part of the TRAF2 molecule, amino-acids 1 to 221, that included the Ring finger and the zink finger motifs. The second construct included only the C-terminal part of the molecule, amino acids 222 to 501, covering the "TRAF-domain" and additional 42 amino acids. These two constructs were served as baits in two hybrid tests. The results clearly show that while clones 9, 10 and 15 did not interact with the construct comprising amino acids 1 to 221 of TRAF2 molecule, they all did bind to the C-terminal construct comprising the "TRAF domain" with the same efficiency as they bound to the full length TRAF2 molecule.

Table II: Summary of the results of the two hybrid screen using TRAF2 as a "bait", in which clones 9, 10 and 15 were picked up.

	Growth on	Color test (min.)	ID/name of clone, as defined by its sequencing.	Number of independent clones
20	50 mM 3AT			
	+++	10 min	TRAF2	150
	++	20 min	new clone number 9	6
25	+++	15 min	new clone number 10	2
	++++	10 min	Lymphotoxin beta receptor	2
	+	15 min	new clone number 15	5

Table III : **Specificity tests**
(interaction with irrelevant baits in the two-hybrid test)

	<u>clone:</u>	clone 9	clone 10	clone 15
5	<u>bait</u>			
	LAMIN	-	-	+
	cyclin D	-	-	+
	p75-IC	-	-	-
	p55-IC	-	-	-
10	MORT1	-	-	-
	TRAF2	+++	+++	+++

Applying several PCR steps to cDNA clone 10, the full length cDNA was cloned from cDNA libraries obtained from RNA of human tissues. This protein was designated NIK for 'NF- κ B inducing kinase' due to the fact that it contains a protein-kinase region (see below). It should be noted that the sequence of clone 10, when initially analyzed (before the obtention of NIK by PCR) was seen to encode for a protein, originally designated NMPI (see co-owned, co-pending IL 117800). This NMPI or clone 10 encoded protein was seen to have sequences corresponding to the I to XI conserved motifs that characterize Ser/Thr protein kinases.

Example 2: Sequencing of new clones

Three of the novel cDNA clones (clones 9, 10 and 15) were purified, amplified in E. Coli and their DNA was subject to sequence analysis. All three clones were found to be partial cDNA clones.

The total lengths of clones 9, 10 and 15 were around 2000, 2700 and 1300 base pairs, respectively.

Figs. 3 and 5 show the sequenced part of clones 9 and 15 and Fig. 4 shows the full sequence of clone 10 :

Figs. 5a-b show the entire nucleotide sequence of clone 15 sequenced from both 5' and 3' ends (a) and the deduced amino acids encoded thereby (b). Clone 15, which is a partial cDNA clone, was found to encode a 172 amino acid long protein.

Clones 9 and 15 are partial clones, which lack their most 5' end of the coding DNA sequences. The deduced amino acid sequences shown in Figs. 3b, 4b and 5b, are all started from the first nucleotide of the respective clone.

The sequence of clone 10 (a partial cDNA clone) which was most thoroughly analyzed, encodes for a protein called NMP1 as noted above, containing Ser/Thr protein kinase motifs. The full length cDNA clone obtained from PCR using the clone 10 as noted above revealed the new TRAF2-binding kinase NIK as mentioned above.

The full nucleotide sequence and its deduced amino acid sequence of NIK are shown in Fig. 6 in which the initiator ATG at nucleotide no. 232 is underlined, and in which the stop codon at nucleotide no. 3073 is indicated by a star. The fully sequenced NIK clone of Fig. 6 is 4596 nucleotides in length within which the NIK coding sequence is contained, this coding for a NIK protein of 947 amino acid residues.

Databank searches revealed that the new amino acid sequence of NIK shows particularly high homology to a group of kinases of which several are known to serve as MAP kinase kinase.

Fig. 7 shows the alignment of :

mouse MEKKK (S1),
BYR2 (S2),
Tpl-2 (S3),
Ewing's sarcoma oncogene (S4),
SS3 (S5),
(STE11) (S6),
(NPK1) (S7),
(BCK1) (S8), and
(NIK) (S9).

Some of those kinases have been identified by virtue of oncogene activity that they possess when in mutated form.

Example 3: Expression of cloned cDNAs and their Co-immunoprecipitation with TRAF2

HeLa-Bujard cells were transfected with TRAF2 tagged with FLAG in pUHD10-3 based expression vector and constructs containing ORF of either clone 9, 10 or 15 fused to HA epitope, as described in Materials and Methods (iv). Cells were then grown for 24 hrs. in Dulbecco's Modified Eagle's Medium (DMEM) plus 10% calf serum with added ^{35}S -Methionine and ^{35}S -Cysteine. At the end of that incubation time cells were lysed in radioimmune precipitation buffer (10 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1% Nonident P-40, 1% deoxycholate, 0.1% SDS, and 1 mM EDTA; 1 ml/ 5×10^5 cells), and the lysate was precleared by incubation with irrelevant rabbit antiserum and Protein G-Sepharose beads (Pharmacia, Sweden). Immunoprecipitation was performed by 1 hour incubation at 4°C of aliquots of the lysate with anti-FLAG (purchased from Eastman Kodak Co.) or anti-HA (clone 12CA5 (Field, J. et al. (1988)) monoclonal antibodies. The expressed proteins were analysed on SDS-PAGE gel followed by autoradiography.

The results of such experiments demonstrated that the partial cDNA clones 9, 10 and 15 encoded proteins of molecular weights around 50-65, 45 and 26 kDa respectively.

No interaction of clone 15 with TRAF2 could be detected, but the proteins encoded by clones 9 and 10 (NIK) as well as the full length NIK, were co-immunoprecipitated with the TRAF2 protein. Samples of cells that were co-transfected with TRAF2 and either one of these two clones and immunoprecipitated with either anti-FLAG or anti-HA antibodies followed by analysis on SDS-PAGE as described above, displayed three bands in each lane; one band corresponding to either clone 9 or 10 encoded proteins and the other two is a doublet of 42 and 44 kDa corresponding to TRAF2 protein.

Example 4: Functional tests

NIK was found to have NF- κ B induction by gel retardation assay. Typically 0.5-1 x 10^6 293 EBNA cells were transfected with either 10 μg of clone 10 in pcDNA3 (Fig. 7 lane 1), 3 μg of pcDNA3 containing cDNA for the p75 TNF receptor (Fig. 7 lane 3), or with both clone 10 (10 μg) and p75 TNF receptor (3 μg) in Fig. 7 lane 2. In each one of the transfections the total amount of transfected DNA was brought to 15 μg with the "empty" pcDNA3 vector. As a control serve 293 EBNA cells transfected with 15 μg pcDNA3 vector alone (Fig. 7 lane 4). Cells were grown for 24 hrs in DMEM medium + 10% calf

serum, then were harvested and treated according to Schreiber et al. (Schreiber, E. et al. (1989). Samples were run on 5 % polyacrylamide gel. NF- κ B was monitored using a set of 32 P-radiolabelled oligonucleotides corresponding to the NF- κ B binding site as probes. (The probes were GATGCCATTGGGGATTCTCTTT and CAGTAAAGAGGAAATCCCCAATGG).

As shown in Table IV NIK induced NF- κ B even more effectively than TRAF-2. On the other hand, clone 10 did not have this effect at all.

Reporter gene assay was performed as follows :

293 EBNA cells were co-transfected with the pcDNA3 vector containing HIV LTR linked to the luciferase reporter gene, together with either pcDNA3 plasmid containing cDNA for the p75 TNF receptor alone, pcDNA3 plasmid containing clone 10 cDNA alone, or with pcDNA3 plasmid containing cDNA for the p75 TNF receptor and a pcDNA3 plasmid listed in Tables IV and V.

The results shown in Table V demonstrate :

a) that clone 10 transfection does not activate NF- κ B induction, while NIK strongly does,

b) that clone 10 as well as NIK in which the active site lysine was replaced with alanine (NIK*) strongly inhibited NF- κ B induction by the cDNA listed in the first column of Table IV.

Deletion of the 3' UTR of NIK (NIK-3'UTR) greatly increased its expression and consequently its ability to block NF- κ B induction when expressed in the mutated form.

Table IV

Activation of NF- κ B by NIK. Gel-retardation assay. Numbers are counts of radioactivity decay events as detected by 'phosphorimager' plate.

transfected cDNA	counts	area (mm ²)
empty vector	327	70.7
TRAF2	3411	70.7
NIK	6532	70.7
clone 10	343	70.7

Table V

Dominant-negative effect of clone 10, NIK K->A mutant on induction of NF- κ B by overexpression of TRAF2, TRADD, MORT1/FADD, TNFR-i, TNFR-II, TNFR-I/FAS chimera, RIP and activation of NF- κ B by NIK. Luciferase test.

Inducer of NF- κ B	empty vector	NIK	NIK-3'UTR	clone 10	NIK*	NIK*-3'UTR	TRAF2 225-501 aa
TRAF2	300	1000		25	30		ND
TRADD	300	800	1000	100	100	5	ND
MORT1/FADD	300	1000		25	80		90
TNFR-I	200	800	1000	50	100	5	ND
TNFR-II	200	750	800	20	90	6	ND
FAS chimera	300	1200		25	50		30
RIP	300	800		75	50		ND
NIK	500			100		10	ND
TNF	200			80			
RelA	1000	ND	ND	1000	ND	ND	ND

Example 5 : Additional characteristics of NIK

In addition to the specificity tests of Example 2 above, further two-hybrid testing of the binding properties of NIK revealed (results not shown) that the initially isolated partial clone of NIK (NIK 624-947) binds specifically to the C-terminal region of TRAF2 (C-TRAF domain), while, in contrast, the full-length NIK bound to both the C-TRAF domain and a region upstream of it (N-TRAF domain). NIK also does not bind to TRAF3. Further, a chimeric molecule containing the C-TRAF domain of TRAF2 and the N-terminal portion of TRAF3 could bind the partial NIK molecule (NIK 624-947) but not the full-length NIK indicating that the binding of full-length NIK to TRAF2 requires both the C-TRAF and N-TRAF domains of TRAF2.

Moreover, NIK does not self-associate, nor does it bind to the intracellular domains of the : p55 and p75 TNF receptors; the CD40 receptor (a member of the TNF/NGF receptor family); and the FAS/APO1 (CD95 receptor). NIK also does not bind to the intracellular proteins associated with these receptors, such as for example TRADD, MORT1 and RIP. These results correlate with those shown in Table II above concerning the binding specificities of the proteins encoded by clones 9, 10 and 15. The various interactions between the various receptors and proteins are depicted schematically in Figs. 2a and 2b, Fig. 2b being more complete.

Northern blot analysis revealed that there is a single transcript of NIK expressed in various tissues at different levels, which transcript has a size of about 5000 nucleotides which is essentially the same as the cloned NIK cDNA (as noted above, see Fig. 6).

Furthermore, as noted above in respect of the protein encoded by clone 10 (originally designated NMPI), the full-length NIK protein also has a serine/threonine protein kinase motif similar to several MAP kinase kinases (MAPKKK) as also arises from the sequence alignments shown in Fig. 7.

In vitro testing of NIK kinase activity revealed that NIK can be autophosphorylated, but not when the active-site lysine and adjacent lysine are replaced with alanine (NIK analog or mutein designated NIK KK429-430AA indicating that the lysines in positions 429 and 430 are replaced with alanines). This also correlates with the above results set forth in Example 4 and shown in Table IV with respect to the NIK* mutein.

As mentioned above, overexpression of NIK in 293 EBNA cells induced NF- κ B to an even greater extent than overexpression of TRAF2, but overexpression of the partial NIK (NIK 624-947) did not bring about NF- κ B activation. In addition, the above noted NIK analog/mutein NIK KK429-430AA also did not bring about NF- κ B activation when overexpressed in these cells. Thus, induction of NF- κ B by NIK depends on an intact kinase function of NIK. In contrast, RIP (see Figs. 2a, b) which also has a kinase domain can still induce NF- κ B activation when its kinase activity is abolished by mutation.

The activation of NF- κ B upon overexpression of NIK was indistinguishable from that produced by treating the cells with TNF, and as with TNF or TRAF2 overexpression, the principal components of NIK-activated NF- κ B were p50 and p65. NIK overexpression caused the degradation of I κ B α and blocking this degradation with N-acetyl-Leu-Leu-

norleucinol (ALLN) resulted (as with TNF) in the accumulation of I κ B molecules having slower SDS-PAGE migration indicative of phosphorylated I κ B α .

Other tests have revealed that NF- κ B can be activated in 293-EBNA cells by TNF as well as by overexpression of p55 and p75 TNF receptors, or overexpression of a p55 TNF receptor in which the intracellular domain of the p55 TNF receptor is replaced by that of the FAS/APO1 receptor. NF- κ B can also be activated by overexpression of TRAF2, TRADD, RIP or MORT1, but not by a MORT1 deletion mutant lacking the region upstream of the 'death domain' of MORT1. As noted above, full length NIK, but not the NIK mutein NIK KK429-430AA nor the partial NIK (NIK 624-947), induces NF- κ B activation. Moreover, expression of the NIK KK429-430AA mutein or NIK 624-947 in 293-EBNA cells together with any of the other above noted agents, i.e. the receptors or associated proteins resulted in the blocking of induction of NF- κ B activation by all of these agents, indicating that NIK activity is directly involved in this NF- κ B induction. Likewise the above observed inhibition by inactive NIK molecules correlates with less I κ B reduction.

NF- κ B is also activated by IL-1 (see scheme in Fig. 2b). This effect is apparently independent of TRAF2 (IL-1 does not bind TRAF2 and the IL-1 effect is not blocked by the expression of a TRAF2 dominant-negative mutant). However, this IL-1 effect is inhibited by the expression of NIK mutants. In addition, the NF- κ B activity observed upon overexpression of the p65 Rel homologue in 293-EBNA cells was unaffected by co-expression of kinase-deficient NIK mutants, indicating that NIK does not affect the function of Rel proteins directly, but participates in their receptor-induced activation.

The cytotoxic activity of TNF (apparently mediated by MORT1-associated protease MACH - see Fig. 2b) is subject to negative regulation by some NF- κ B-inducible genes. The antagonizing consequences of NF- κ B-mediated gene induction and MACH activation may explain why TNF itself, as well as IL-1 can induce cellular resistance to TNF cytotoxicity. In line with this, it has also been found in accordance with the present invention that the expression of NIK dominant-negative mutants in 293-EBNA cells significantly increased their susceptibility to killing by TNF, and that overexpression of native (full-length, wild-type) NIK inhibited the killing of the cells by TNF or by overexpression of the p55 TNF receptor (this receptor has an intracellular domain containing a 'death domain' region that when expressed in cells, in the absence of any TNF, can induce on its own cell cytotoxicity

- see above referred-to publications of present inventors and co-owned, co-pending applications).

Example 6 : Further functional tests for NIK biological activity

In accordance with the present invention, it has also been found that expression of NIK dominant-negative mutants could also block the induction of NF- κ B activation in 293-EBNA cells by other inducing agents including : (i) the well known bacterial endotoxin, lipopolysaccharide (LPS); (ii) a well known forbol myristate acetate, which is a known protein kinase C activator; and (iii) the HTLV-1 protein TAX.

Furthermore, the expression of dominant-negative mutants of NIK in the 293-EBNA cells has been found to have essentially no effect on the TNF-induced activation of the Jun kinase indicating that NIK acts in a specific and possibly direct manner to enhance the phosphorylation of I κ B without affecting the MAP kinases involved in Jun phosphorylation.

In view of all of the above mentioned it arises that the kinase activity of NIK is part of a signaling cascade that is responsible for NF- κ B activation and which cascade is common to the two TNF receptors, the FAS/APO1 receptor and the IL-1-receptor. NIK appears to play a specific role in this cascade. The binding of NIK to TRAF2 may serve to enable NIK to be affected by both the TNF receptors and the FAS/APO1 receptor. By analogy to the MAP kinase cascades, NIK may serve as a substrate for a kinase (MAPKKKK) upon being recruited by TRAF2 to the stimulated receptors, so that when NIK is phosphorylated it phosphorylates and activates other kinases (or may induce directly NF- κ B activation by direct phosphorylation of I κ B). The IL-1-induced NF- κ B activation is independent of TRAF2 and hence the activation of NIK by the IL-1-receptor may be mediated by another protein IRAK, a serine/threonine kinase that is recruited to the IL-1 receptor after stimulation (Cao et al., 1996b), and also by TRAF6 which binds IRAK (see Cao et al., 1996a, as well as scheme in Fig. 2b). As noted above, the target of NIK, or of a cascade of kinases activated by it, is likely to be I κ B. NIK may also phosphorylate TRAF proteins or regulatory proteins that bind to them for example TANK-1/TRAF (see Cheng and Baltimore, 1996; Rothe et al., 1996) creating docking sites for other proteins.

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CLAIMS:

1. A DNA sequence encoding a protein capable of binding to a tumor necrosis factor receptor-associated factor (TRAF) molecule.

5 2. A DNA sequence according to claim 1, wherein the TRAF molecule is TRAF2.

3. A DNA sequence according to claim 2, wherein said encoded protein binds to at least the 222-501 amino acid sequence of TRAF2.

4. A DNA sequence according to any one of claims 1 to 3, selected from the group consisting of:

10 (a) a cDNA sequence of the herein designated clone 9 comprising the nucleotide sequence depicted in Fig 3a.;

(b) a cDNA sequence of the herein designated clone 10 comprising the nucleotide sequence depicted in Fig 4;

15 (c) a cDNA sequence of the herein designated clone 15 comprising the nucleotide sequence depicted in Fig. 5a;

(d) a fragment of a sequence (a)-(c) which encodes a biologically active protein capable of binding to least the 222-501 amino acid sequence of TRAF2;

20 (e) a DNA sequence capable of hybridization to a sequence of (a)-(d) under moderately stringent conditions and which encodes a biologically active protein capable of binding to at least the 222-501 amino acid sequence of TRAF2; and

(f) a DNA sequence which is degenerate as a result of the genetic code to the DNA sequences defined in (a)-(e) and which encodes a biologically active protein capable of binding to at least the 222-501 amino acid sequence of TRAF2.

25 5. A DNA sequence according to any one of claims 1 to 4, selected from the sequences contained in the herein designated cDNA clones 9 and 15.

6. A DNA sequence according to any one of claims 1 to 4, which DNA encodes a protein that also modulates NF- κ B activity.

7. A DNA sequence according to claim 6, selected from the sequences contained in the herein designated cDNA clone 10.

30 8. A DNA sequence according to claim 1 or 6, comprising the DNA sequence encoding the protein NIK (as herein defined).

9. A DNA sequence encoding the protein NIK, isoforms, fragments or analogs thereof, said NIK, isoforms, fragments or analogs thereof being capable of binding to TRAF2 and which is capable of modulating the activity of NF- κ B.

10. A DNA sequence according to claim 9, selected from the group consisting of:

5 a) a cDNA sequence derived from the coding region of a native NIK protein;

b) DNA sequences capable of hybridization to a sequence of (a) under moderately stringent conditions and which encode a biologically active NIK; and

10 c) DNA sequences which are degenerate as a result of the genetic code to the sequences defined in (a) and (b) and which encode a biologically active NIK protein.

11. A DNA sequence according to claim 9 or 10 comprising at least part of the sequence depicted in Fig. 6 and encoding at least one active NIK protein, isoform, analog or fragment.

12. A DNA sequence according to claim 11 encoding a NIK protein, isoform, 15 analog or fragment having at least part of the amino acid sequence depicted in Fig. 6.

13. A vector comprising a DNA sequence according to any one of claims 1-12.

14. A vector according to claim 13 capable of being expressed in a eukaryotic host cell.

15. A vector according to claim 13 capable of being expressed in a prokaryotic 20 host cell.

16. Transformed eukaryotic or prokaryotic host cells containing a vector according to any one of claims 13-15.

17. A TRAF-binding protein, isoforms, fragments, analogs and derivatives thereof, encoded by a DNA sequence according to any one of claims 1-12, said protein, isoforms, 25 fragments, analogs and derivatives thereof being capable of binding to at least the portion of the TRAF2 protein between amino acids 222-501 of TRAF2.

18. A protein according to claim 17 being the protein encoded by herein designated clone 10.

19. A protein, isoforms, fragments, analogs and derivatives thereof, according to 30 claim 17 being the NIK protein, isoforms, analogs, fragments and derivatives thereof encoded by the DNA sequence according to any one of claims 1-12.

20. A NIK protein, isoforms, analogs, fragments and derivatives thereof according to claim 19, wherein said protein, isoforms, fragments and derivatives have at least part of the amino acid sequence depicted in Fig. 6.

21. A method for producing a protein, isoform, fragment, analog or derivative thereof according to any one of claims 17-19, which comprises growing a transformed host cell according to claim 16 under conditions suitable for the expression of said protein, isoform, fragment, analog or derivative thereof, effecting post-translational modification, as necessary, for obtaining said protein, isoform, fragment, analog or derivative thereof, isolating said expressed protein, isoform, fragment, analog or derivative.

22. Antibodies or active fragments or derivatives thereof, specific for the TRAF-binding protein, isoform, analog, fragment or derivative thereof according to claim 17 or 18; or specific for the NIK protein, isoform, analog, fragment or derivative thereof according to claim 19 or 20.

23. A method for the modulation or mediation in cells of the activity of NF- κ B or any other intracellular signaling activity modulated or mediated by TRAF2 or by other molecules to which a protein, isoform, analog, fragment or derivative thereof according to any one of claims 17-20 binds, said method comprising treating said cells by introducing into said cells one or more of said protein, isoform, analog, fragment or derivative thereof in a form suitable for intracellular introduction thereof, or introducing into said cells a DNA sequence encoding said one or more protein, isoform, analog, fragment or derivative thereof in the form of a suitable vector carrying said sequence, said vector being capable of effecting the insertion of said sequence into said cells in a way that said sequence is expressed in said cells.

24. A method according to claim 23, wherein said treating of cells comprises introducing into said cells a DNA sequence encoding said protein, isoform, fragment, analog or derivative in the form of a suitable vector carrying said sequence, said vector being capable of effecting the insertion of said sequence into said cells in a way that said sequence is expressed in said cells.

25. A method according to claim 23 or 24 wherein said treating of said cells is by transfection of said cells with a recombinant animal virus vector comprising the steps of:

(a) constructing a recombinant animal virus vector carrying a sequence encoding a viral surface protein (ligand) that is capable of binding to a specific cell surface receptor on

the surface of said cells to be treated and a second sequence encoding a protein selected from the said protein, isoforms, analogs, fragments and derivatives according to any one of claims 17-20, that when expressed in said cells is capable of modulating/mediating the activity of NF- κ B or any other intracellular signaling activity modulated/mediated by

5 TRAF2 or other said molecules; and

(b) infecting said cells with said vector of (a).

26. A method for modulating TRAF2 modulated/mediated effect on cells comprising treating said cells with antibodies or active fragments or derivatives thereof, according to claim 22, said treating being by application of a suitable composition
10 containing said antibodies, active fragments or derivatives thereof to said cells, wherein when the TRAF2-binding protein or portions thereof of said cells are exposed on the extracellular surface, said composition is formulated for extracellular application, and when said TRAF2-binding proteins are intracellular said composition is formulated for intracellular application.

27. A method for modulating the TRAF2 modulated/mediated effect on cells comprising treating said cells with an oligonucleotide sequence encoding an antisense sequence for at least part of the DNA sequence encoding a TRAF2-binding protein according to any one of claims 1-11, said oligonucleotide sequence being capable of
15 blocking the expression of the TRAF2-binding protein.

28. A method according to claim 27 wherein said oligonucleotide sequence is introduced to said cells via a virus of claim 25 wherein said second sequence of said virus encodes said oligonucleotide sequence.
20

29. A method for modulating the TRAF2 modulated/mediated effect on cells comprising applying the ribozyme procedure in which a vector encoding a ribozyme sequence capable of interacting with a cellular mRNA sequence encoding a TRAF2-binding
25 protein according to any one of claims 17-20, is introduced into said cells in a form that permits expression of said ribozyme sequence in said cells, and wherein when said ribozyme sequence is expressed in said cells it interacts with said cellular mRNA sequence and cleaves said mRNA sequence resulting in the inhibition of expression of said TRAF2-binding protein in said cells.
30

30. A method for isolating and identifying proteins, according to any one of claims 17-20, capable of binding directly to TRAF2, comprising applying the yeast two-hybrid

procedure in which a sequence encoding said TRAF2 is carried by one hybrid vector, and sequence from a cDNA or genomic DNA library is carried by the second hybrid vector, the vectors then being used to transform yeast host cells and the positive transformed cells being isolated, followed by extraction of the said second hybrid vector to obtain a sequence
5 encoding a protein which binds to said TRAF2.

31. A method according to any one of claims 23-30 wherein said protein is NIK or at least one of the NIK isoforms, analogs, fragments and derivatives thereof.

32. A pharmaceutical composition for the modulation of the TRAF2 modulated/mediated effect on cells comprising, as active ingredient at least one TRAF2-
10 binding proteins, according to any one of claims 17-20, its biologically active fragments, analogs, derivatives or mixtures thereof.

33. A pharmaceutical composition for modulating the TRAF2 modulated/mediated effect on cells comprising, as active ingredient, a recombinant animal virus vector encoding a protein capable of binding a cell surface receptor and encoding at least one TRAF2-
15 binding protein, isoform, active fragments or analogs, according to any one of claims 17-20.

34. A pharmaceutical composition for modulating the TRAF2 modulated/mediated effect on cells comprising as active ingredient, an oligonucleotide sequence encoding an anti-sense sequence of the TRAF2-binding protein mRNA sequence according to any one
20 of claims 1-11.

35. A pharmaceutical composition for the prevention or treatment of a pathological condition associated with NF- κ B induction or with any other activity mediated by TRAF2 or by other molecules to which a protein according to any one of claims 17-20 binds, said composition comprising an effective amount of a protein encoded by clone 10 or a DNA
25 molecule coding therefor, or a molecule capable of disrupting the interaction of said protein encoded by clone 10 with TRAF2 or any other molecule to which a protein encoded by clone 10 binds.

36. A pharmaceutical composition for the prevention or treatment of a pathological condition associated with NF- κ B induction or with any other activity mediated by TRAF2
30 or by other molecules to which a protein according to any one of claims 17-20 binds, said composition comprising an effective amount of a NIK protein, isoform, fragment, analog or derivative thereof, or a DNA molecule coding therefor, or a molecule capable of disrupting

the interaction of said NIK protein, isoform, fragment, analog or derivative thereof with TRAF2 or any other molecule to which said NIK protein, isoform, fragment, analog or derivative binds.

37. A pharmaceutical composition for the prevention or treatment of a pathological condition associated with NF- κ B induction or with any other activity mediated by TRAF2 or by other molecules to which the protein NIK binds, said composition comprising a molecule capable of interfering with the protein kinase activity of NIK.

38. A pharmaceutical composition for the prevention or treatment of a pathological condition associated with NF- κ B induction or with any other activity mediated by TRAF2 or by other molecules to which a protein encoded by clone 10 according to claim 18 binds, said composition comprising an effective amount of a protein encoded by clone 10 or a DNA molecule coding therefor, or a molecule capable of disrupting the interaction of said protein encoded by clone 10 with TRAF2 or any other molecule to which said protein encoded by clone 10 binds.

39. A pharmaceutical composition for the prevention or treatment of a pathological condition associated with NF- κ B induction or with any other activity mediated by TRAF2 or by other molecules to which a NIK protein, isoform, fragment, analog or derivative according to claim 19 or 20 binds, said composition comprising an effective amount of a NIK protein, isoform, fragment, analog or derivative thereof, or a DNA molecule coding therefor, or a molecule capable of disrupting the interaction of said NIK protein, isoform, fragment, analog or derivative thereof with TRAF2 or any other molecule to which said NIK protein, isoform, fragment, analog or derivative binds.

40. A method for the prevention or treatment of a pathological condition associated with NF- κ B induction or with any other activity mediated by TRAF2 or by other molecules to which a protein according to any one of claims 17-20 binds, said method comprising administering to a patient in need an effective amount of a protein or isoform, fragment, analog and derivative thereof or a mixture of any thereof according to any one of claims 17-20, or a DNA molecule coding therefor, or a molecule capable of disrupting the interaction of said protein or isoform, fragment, analog and derivative thereof or a mixture of any thereof according to any one of claims 17-20 with TRAF2 or any other molecule to which said protein or isoform, fragment, analog and derivative thereof or a mixture of any thereof according to any one of claims 17-20 binds.

41. A method according to claim 40 wherein said protein is encoded by clone 10.

42. A method according to claim 40, wherein said protein is NIK.

43. A method for screening of a ligand capable of binding to a protein according to any one of claims 17-20 comprising contacting an affinity chromatography matrix to which said protein is attached with a cell extract whereby the ligand is bound to said matrix, and eluting, isolating and analyzing said ligand.

44. A method for screening of a DNA sequence coding for a ligand capable of binding to a protein according to any one of claims 17-20 comprising applying the yeast two-hybrid procedure in which a sequence encoding said protein is carried by one hybrid vector and sequences from a cDNA or genomic DNA library are carried by the second hybrid vector, transforming yeast host cells with said vectors, isolating the positively transformed cells, and extracting said second hybrid vector to obtain a sequence encoding said ligand.

45. A method for identifying and producing a ligand capable of modulating the cellular activity modulated/mediated by TRAF2 comprising :

a) screening for a ligand capable of binding to a polypeptide comprising at least a portion of TRAF2 having the amino acid residues 222-501 of TRAF2;

b) identifying and characterizing a ligand, other than TRAF2 or portions of a receptor of the TNF/NGF receptor family, found by said screening step to be capable of said binding; and

c) producing said ligand in substantially isolated and purified form.

46. A method for identifying and producing a ligand capable of modulating the cellular activity modulated or mediated by a protein according to any one of claims 17-20 comprising :

a) screening for a ligand capable of binding to a polypeptide comprising at least a portion of the NIK sequence depicted in Fig. 6;

b) identifying and characterizing a ligand, other than TRAF2 or portions of a receptor of the TNF/NGF receptor family, found by said screening step to be capable of said binding; and

c) producing said ligand in substantially isolated and purified form.

47. A method for identifying and producing a ligand capable of modulating the cellular activity modulated/mediated by NIK comprising :

72

a) screening for a ligand capable of binding to at least a portion of the NIK sequence depicted in Fig. 6;

b) identifying and characterizing a ligand, other than TRAF2 or portions of a receptor of the TNF/NGF receptor family, found by said screening step to be capable of said binding; and

c) producing said ligand in substantially isolated and purified form.

48. A method for identifying and producing a molecule capable of directly or indirectly modulating the cellular activity modulated/mediated by NIK, comprising :

a) screening for a molecule capable of modulating activities modulated/mediated by NIK;

b) identifying and characterizing said molecule; and

c) producing said molecule in substantially isolated and purified form.

49. A method for identifying and producing a molecule capable of directly or indirectly modulating the cellular activity modulated/mediated by a protein according to any one of claims 17-20, comprising :

a) screening for a molecule capable of modulating activities modulated/mediated by a protein according to any one of claims 17-20;

b) identifying and characterizing said molecule; and

c) producing said molecule in substantially isolated and purified form.

ABSTRACT

A DNA sequence encoding a protein capable of binding to a tumor necrosis factor receptor-associated factor (TRAF) molecule, TRAF-binding proteins, their isoforms, analogs, fragments and derivatives encoded by the DNA sequence, their methods for the production of the DNA sequences and proteins, and the uses for the DNA sequence and proteins.

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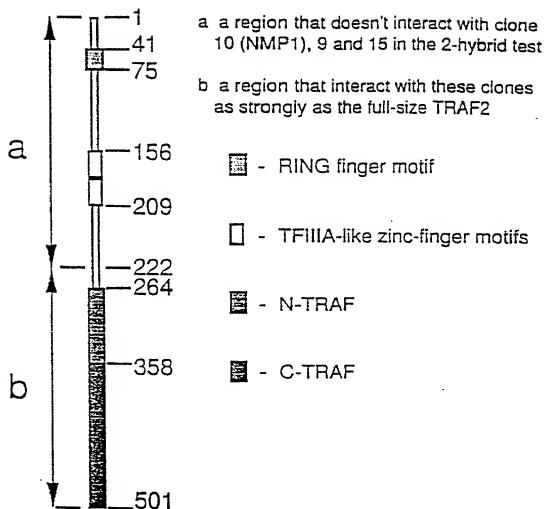


FIG. 1

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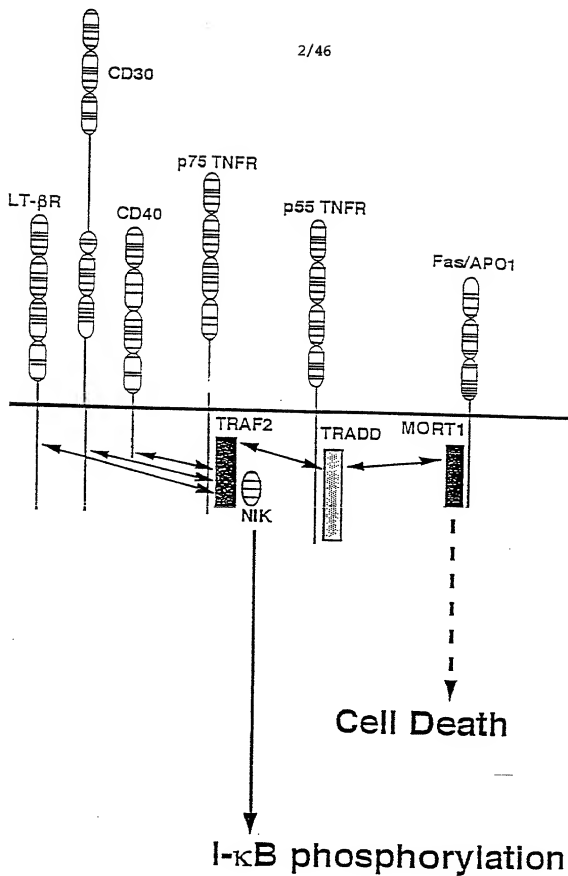


FIG. 2A

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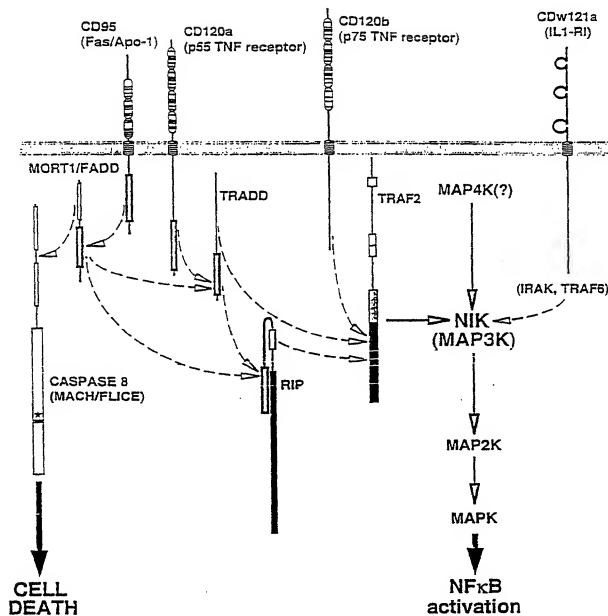


FIG. 2B

4/46

Length: 1906 July 7, 1996 12:35 Type: N Check: 7122 ..

1 CATTGGGTCA CGCGGTGGCG GCGCTCTAGA ATAGTGGATC CCCCGGGCTG
51 CAGGAATTCG ATTCGAGGCC ACGAAGGCCG GCGGCGCGGC GCAnGCACCG
101 GCGCGGGGAn AGGCnCCATG AGCGGATCnC nGAACnATGA CAAAAGACAA
151 TTTCTGCTGG AGCGACTGCT GGATGCAGTG AAACAGTGCC AGATCCGCTD
201 TnGAGGGAGA AAGGAGATTG CCTCGGATTG CGACAGCAGG GTCACCTGTC
251 TGTGTGCCCA GTTTGAAGCC GTCCTGCAGC ATGGCTTGAA GAGGAGTCGA
301 GGATTGGCAC TCACAGCGGC AGCGATCAAG CAGGCAGCGG GCTTTGCCAG
351 CAAAACCGAA ACAGAGCCCCG TGTTCTGGTA CTACGTGAAG GAGTCCTCA
401 ACAAGCACGA GCTGCAGCGC TTCTACTCCC TGCGCCACAT CGCCTCAGAC
451 gTGGGCGGGG GTCGCGCCTG GCTGcGCTGT GCCCTCAACG AACACTCCCT
501 GGAGCGCTAC CTGCACATGC TCCTGGCCGA CCGCTGCAGG CTGAGCACTT
551 TTTATGAAGA CTGGTCTTTT GTGATGGaTG AAGAAAGGTC CAGTATGCTT
601 CCTACCATGG CAGCAGGTCT GAACTCCATA CTCCTTGCGA TTAACATCGA
651 CAACAAGGAT TTGAACGGGC AGAGTAAGTT TGCTCCCACC GTTTCAGACC
701 TCTTAAAGGA GTCAACGCAG AACGTGACCT CCTTGCTGAA GGAGTCCACG
751 CAAGGAGtGA GCAGCCTGTT CAGGGAGATC ACAGcCTcCT cTGCcGTcTC
801 CATCcTCATC AAACCTGAAC AGGAGACCGA CCCTTGCCGTG TCGTGTCCAG
851 GAATGTCAST GCTGATGCCA AATGCAAAAA GGAGCGGAAG AAGAAAAAGA

FIG. 3A

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901 AAGTGACCAA CATAATCTCA TTTGATGATG AGGAAGATGA GCAGAACTCT
951 GGGGACGTGT TTAAAAAGAC ACCTGGGGCA GGGGAGAGCT CAGAGGACAA
1001 CTCCGACCGC TCCTCTGTCA ATATCATGTC CGCCTTTGAA AGCCCCCTCG
1051 GGCCTAACTC CAATGGAATC AGAGCAGCAA CTCATGGAAA ATTGATTCCC
1101 TGTCTTTGAA CGGGGAGTTT GGGTACCAGA AGCTTGATGT GAAAAGCATC
1151 GATGATGAAG ATGTGGATGA AAACGAAGAT GACGTGTATG GAAACTCATC
1201 AGGAAGGAAG CACAGGGGCC ACTCGGAGTC GCCCAGAGAAG CCACTGSAAG
1251 GGAACACCTG CCTCTCCcAG ATGCACAGCT GGgCtCCGCT GAAGgTgCTG
1301 CaCaATGACT CCGACATCCT CTTCCCTGTC AGTGGCGTGG gCTCCTACAG
1351 CCCAGCAGAT gCCCCCCTCG GAAGCCTGGA GAACGGGACA GGRCCAGAGG
1401 ACCACGTTCT CCCGGATCCT GGACTTCGGT ACAGTGTGGA AGCCAGCTCT

FIG. 3B

1451 CCAGGCCACG GAAGTCCTCT GAGCAGCCTG TTACTTCTGC CTCAGTGCCA
1501 GAGTCCATGA CAATTAGTGA ACTGCGCCAG GCCACTGTGG CCATGATGAA
1551 CAGGAAGGAT GAGCTGGAGG AGGAGAACAG ATCACTGCGA AACCTGCTCG
1601 ACGGTGAGAT GGAGCACTCA GCCGCGCTCC GGCAAGAGGT GGACACCTTG
1651 AAAAGGAAGG TGGCTGAACA GGAGGAGCGG CAGGGCATGA AGGTCCAGGC
1701 GCTGGCCAGC TATCTTTGCT ATTTTGTGAG GAGATTCTAA CCCCACGTGA
1751 GAACCATGTG GTGGAGAAAT GGAGGGAGAG AGAAATCCAA CAGTTCCTGA
1801 TAGTCTCATT TGAGCTCCTG GATCCAGTCT TTCCTGAAGC TGTGTTTCCT
1851 CTGGACTTTT CATGTATGTG AGCCAATAAA TTGCTTTCAT TCCTTGAAAA
1901 AAAAAA

FIG. 3C

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TRANSLATE of: 9hhh check: 7122 from: 1 to: 1906
generated symbols 1 to: 635.

9hhh.pep Length: 604 August 23, 1996 15:03 Type: P Check: 4554

1 XTGPGXGXMS GSXNXDKRQF LLERLLDAVK QCQIRFXGRK EIASDSDSRV
51 TCLCAQFEAV LQHGLKRSRG LALTAATAIKQ AAGFASKTET EPVFWYYVKE
101 VLNKHELQRF YSLRHIASDV GRGRAWLRCA LNEHSLERYL HMLLADRCRL
151 STFYEDWSFV MDEERSSMLP TMAAGLSIL FAINIDNKDL NGQSKFAPTV
201 SDLLKESTQN VTSLLKESTQ GVSSLFREIT ASSAVSILIK PEQETDFCLS
251 CPGMSVLMPN AKRSGRRKRK *PT*SHIMMR KMSRTLGTCL KRHLGQGRAQ
301 RTTPTAPLSI SCFPLKAPSG LTPMESEQLL MEN*FFVFER GVWVPEA*CE
351 KHR**RCG*K RR*RVWKLIR KEAQGPLGVA REATGREHLP LPDAQLGSAA
401 GAAQ*LREPL PCQWRGLLQP SRCPPRKPGZ RDRTRGPRSP GSWTSVQCGS
451 QLSRPRKSSE QPVTASVPE SMTISELRQA TVAMMNRKDE LEEENRSLRN
501 LLDGEMEHS AALQEVDTLK RKVAEQEERQ GMKVQALASY LCYFVRRF*P
551 HVRTMWWRNG GREKSNSS** SHLSSWISQF LKLCFLWTFH VCEPINCFSH
601 LKKK

FIG. 3D

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clone 10 Length: 2631 August 23, 1996 17:18 Type: N Check: 5107 ..

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1   CCCcTcTcAC AGCcCAGGCC ATCCAAGAGG GgCTGAGGAA AGAGCCCATC
51  cACCGcGTGT cTGCAGcGGA GcTGGGAGGG AAGTGAAACC GGGCAcTACA
101 GCAAAGTGGGA GGTcTGAAGA GCCCTTGGAG GGGAGAATAT AAAGAACCAA
151 GACATCCACc GCCAAATCAA GCCaAtTACC ACCAGACCcT CcATGCCcAg
201 CCGAGAGAGc TtTcGCCAAG GGGCCcAGGG CCCCcGGCCAg CTGAGGAGAC
251 AACAggCAGA GCCCCTAAGc TCCAGCcTCC TcTCCCACCA GAGCCCCCAG
301 AGCCaAACAA GTcTcTCCCC tTgACTtTGA GCAAGGAGGA GTcTGGGATG
351 TGGGAACCCCT TAcTcTcGTC cTCCCTGGAG CCAGCCCCCTG CCAGAACCc
401 CAGcTCACCA GAGCGGAAAG CAACCGTCCC GGAGCAGGAA CTGCAGCAGC
451 TGGAAATAGA ATTATTCCCTC AACAGCCCTGT CCCAGcCATT TtTcTGGAG
501 GAGCAGGAGC AAATTCTcTc GTGCCCTCAGC ATCGACAGCC TCTCCcTGTc
551 GGTATGACAGT GAGAAGAACC CATCAAAGGC CTCTCAAAGC TCGCGGGACA
601 CCCTGAGCTC AGGCGTACAC TCCTGGAGCA GcCAGGCCGA GgCtCGAAGc
651 TCCAGCTGGA ACATGGTGcT GSCCCGGGgG CGcCCcACCG ACACCCcAAG
701 CTATTTCaAT GGTGTGAAAG TCCAATACA GTCTCTTAAT GGTGAACACC
751 TGCACATCCG GGAgtTCCAC CGGGTCAAAG TGGGAGACAT CGCCACTGGC
801 ATCAGCAGCC AGATCCcAGC TGcAGCCCTC AGCTTGGTCA CCAAGAGCGG
851 GCAGCCTGTT CGCTACGACA TGGAGGTGCC AGACTCGGGC ATCGACCTGC
901 AGTGCAcACT GSCCCCTGAT GSCAGCTTCG CcTGGAGCTG GAGGGTCAAG
951 CATGGCCAGC TGGAGAACAG GCCCTAACCC TGCCCTCCAC CGCCGGcTCC
1001 ACACtGCCCG aAAGCAGCCCT TCCTGCTCGG tGCACGATGC TGCCCTGaaa
1051 AcACAGGcTC AGCcGTTCCC AgGGgATyTG .CCAGcCCCC cGgcTcArCa
1101 G. tGGGAaAc AGGGcTcG. CAGC.AGC.A AGGT.gGGGG CAAGC.AGAA
1151 TGcTcTCCAG GATTTCACA. CcTGAGCCC. TGCCCCA.CC cTGcTgaada
1201 AAaAcAyT.CC GCcAcGtGAA GagAcAGaAG GAGGATGG.C AGGAgtT..A
1251 CcTygGGGAA aCaAAAAcAGg gaTcTtT. tT cTGcCCcTGC TCCAGT.cGA

```

FIG. 4A

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1301 gTGGCCTG. ACCCGcTTGG A.TCAgtGAC CATTTGtTGG CAGA.CAGGG
 1351 GagAgCAGcT TCCAGCcTGG gTCAGAAGGG GTGGGcGAGC CcTcGGGCC
 1401 cTcAcCCT.c CAGGcTgcTG tG.AGAGTGT CAAGTgtGTA AGGG.CCCAA
 1451 A.cTcAGG.T TCAGTGCAGA ACCAgGT.CA GCAGGTATGC CCGCCCC.TA
 1501 GGTAA..GG GGGCCcTcT. AAACCCCTTG cCT.GGCC.T. CAcCT.GGCC
 1551 AGCTCA.CCC cTTTTGGGTG TAGGGGAAAA GAATGCCTGA CCCTGGGAAG
 1601 GCTWCCCTGG TAqAATACAC CACACTTTTC AGGTTGTTC AACACAGTc
 1651 CTGAGTTGAC CTCTGGTTCA GCCAAGGACC AAAGAAGGTG TGTAAGTGAA
 1701 GTGGTTCTCA gT.CCCCAgA CATgTgCCCC TTTGCTGCTG GCTACCACTC
 1751 TTCCCCAgAg CAGCAGGcCC CgAgCCCCCTT CAGGcCCAgC AcTgcCCcAG
 1801 AcTCgCTGGC aCTCAGTTCC CTCATCTGTA AAGGTGAAGG GTGATGCAGG

 1851 ATATGCCTGA CAGGAACAGT CTGTGGAtGG AcATGATCAg TGcT.AAGG.
 1901 AAAGCAGcAG AGaGAGACgy TcGGCGCCCC CAg.CCCCAc T.ATCAGTgT
 1951 .CCAqCGTGC T.GGTT.CCC CAg.AGCACA GcT.CAg.CA TcA.CACTGA
 2001 CACT.CAcCC T.GCCcTGCC CCT.GGCCA. GAqGGTACTG CCG.ACGGCA
 2051 CTTTGCAc.T CTGATG.ACC TCAAAGCACT TTCATGgcT. GcCCTcT..G
 2101 GCAGGG.CAG GG.CAGGS.C AgTGAcA.CT GTAgG.AGCA TA.gCAA.GC
 2151 CAqGAGATGG GGTG.AAGGG A.CACAGTCT TGAGCTGTCC A.CATGCATG
 2201 TGAcT.CCTC AAAcCTcTT. .CCAG.ATTT CTCTAAGAAT AGCA.CCCCC
 2251 TT.CCCcATT GCCCCAGCTT AgCCTCTTCT CCCAGGGGAG CTA.CTCAgG
 2301 ACTCAGTAq CATTAAATCA GCTGTG.AAT CGTCAGGGGG TGTCGTCTAg
 2351 CCTCAACCTC CTGGGGCaGG GGACgCCGAg ACTCCGTGGG AqAaGCTCAT
 2401 TcCcAcATCT TGCCAAgACA gCCTTT.GTC CAgCTGTCCA CATTGAgTCA
 2451 gACTGCTCCC GGGGAgAgAg cCCCGGcCCC CAgCACATAA AGAACTGCAG
 2501 CCTTGGTACT GCAGAGTCTG GGTGTAGAG AACTCTTTGT AAGCAATAAA
 2551 GTTTGGGGTG ATGACAAATG TTAaaaaaAG GCCTTCGTGG CCTCGAATCA
 2601 AGCTTATCGA TACCCTCGAC CTCGAGGGGG G

FIG. 4B

SUBSTITUTE SHEET (RULE 26)

10/46

Length: 1253 July 10, 1996 clone15

1 CATTGGAGTC ACGCGGTGGC GGCCTCTAG AATAGTGGAT CCCCgggCTg
51 CA.GGAATTC GATTGAGcC CACGAAGGCC CCTTCTTCTG TGCTCGCGGC
101 ACGTTTACaG CCGCAAGCac CCAGCGGCAg CTGAAGGAGG CTTTGTGAqAG
151 GCTCCTgCCC CAGGTGGAGG CGGCCCCGAA GGCCATCCgC GCCGCTCAGG
201 TGGAGCGCTA TGTGCCCGAA CACGAGCGAT GCTGCTGGTG CCTGTGCTGC
251 GGCTGTGAGG TGCGGGAACA CCTGAGCCAT GGAAACCTGA CGGTGCTGTA
301 CGGGGGgCTG CTGGAGCATC TGGCCAGCCC AGAGCACAAg AAAGCAACCA
351 ACAAATTCTG GTGGGAGAAC AAAGCTGAGG TCCAGATGAA AGAGAAGTTT
401 CTGGTCACTC CCCAGGATTA TGCGCGATTC AAGAAATCCA TGGTGAAAGG
451 TTTGGATTCC TATGAAGAAA AGGAGGATAA AGTGAtCAAG GAgATGgCAG
501 CTCAGATCCG TGaGGTGGAg CAGAgCCGAC AGGAgTGGt TCGGtCTGTc
551 TTAGAgCctC AGGCAGTGcC AGaCCAGAA GAGGGcTCTT CAGCAcCTAG
601 AAGCTGGAAA GSGATGAACA GCCAAGTAGc TTCCAGCTTA CAGcAGcCCT
651 CAaATTtGGA CCTGCCACCA GCTCCAGAGC TTGAcTGGAT GGAAGACAGGA
701 CCATCTCTGA CATTcATTG CCATCAGGAT ATACCAGGAg TTGTaACAT
751 CCACTCAGGT GCCACACCTC CCTGGATGAT CCAAGATGAA GAATACATTG
801 CTGGGAACCA AGAAATAGGA CCATCCTATG AAGAATTTCT TAAAGAAAAG
851 GAAAAACAGA AGTTGAAAAA ACTCCCCCA GACCAGATTG GGGCCAACCTT
901 TGATCACAGC TCCAGGACCA GTGCAGGCTG GCTGCCCTCT TTTgGgcCGC
951 GTCTGGAATA ATGGACGCCG CTGGCAGTCC AGaCATCAAC TcCAAAACTG
1001 AAGCTGCAGC AATGAAGAAG CAGTCACATA CAGAAAAAG CTaATCATGC
1051 TCTCTACCAa CTACCATGAG GCTAAAAGCC AAAGTCAACC AAACCCCTAT
1101 TATACCTTCC ACCCAaATC TTTATCATG TCTTTCTTAG GAAACAGACA
1151 TACTCATTCA TTTGATTAA TAAAGTTTAA TTTTTCGGCC TTCGTGGCCT
1201 CGAATCAAGC TTATCGATAC CGtCGACCTC GAGGGGGGGC CGTACCCACT
1251 TTT

FIG. 5A

SUBSTITUTE SHEET (RULE 26)

11/46

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 generated symbols 1 to: 417.

15cc.pep Length: 417 August 23, 1996 14:32 Type: P Check: 7921 ..

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 101 GGLLEHLASP EHKXATNKFV WENKAQVQMK EXFLVTPQDY ARFKKSMVKG
 151 LDSYEEKEDK VIKEMAAQIR EVEQSRQEVV RSVLEPQAVP DPEEGSSAPR
 201 SWKGMNSQVA SSLQQPSNLD LPPAPELDWM ETGPSLTFFIG HQDIPGVGNI
 251 HSGATPFWMI QDEEYIAGNQ EIGPSYEEFL KEKERQKLKK LPPDRVGANF
 301 DHSSRTSAGW LPSPGPRLE* WTPLAVQTST PKLKLQ*RS SHIQKKANHA
 351 LYQLP*G*KP KSTKPLLYLP PKFFIIVFLR KQYYSFI*FN KVLFFGLRGL
 401 ESSLSIFSTS RGGRTHF

FIG. 5B

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1 AGC GGG ACT GTG CCG TGT GGA AGC TGT AGC TGT TGA AGG TGG ACT CTG TTA CCA TTG
 31
 61 AGG ATG TTT GGA GGA TGA GTA TGT GTG GCA GAG GCA CAC ATA AAC AGG CAG AGA CCC TTT
 91
 121 GCC CCT GCC TTT CTC CCC CAA CCC AAG GCT GAC CTG TGT TCT CCC AGG TCT GGG ATT CTA
 151
 181 AGT GAC CTG CTC TGT GTT TGG TCT CTC TCA GGA TGA GCA CAA GCC TGG GAG ATG GCA GTG
 211
 241 ATG GAA ATG GCC TGC CCA GGT GCC CCT GGC TCA GCA GTG GGG CAG CAG AAG GAA CTC CCC
 271 M A V
 301 M E M A C P G A P G S A V G Q Q K E L P
 331
 AAG CCA AAG GAG AAG ACG CCG CCA CTG GGG AAG AAA CAG AGC TCC GTC TAC AAG CTT GAG
 361 K P K E K T P P L G K K Q S S V Y K L E
 391
 GCC GTG GAG AAG AGC CCT GTG TTC TGC GGA AAG TGG CAG ATC CTG AAT GAC GTG ATT ACC
 421 A V E K S P V F C G K W E I L N D V I T
 451
 AAG GGC ACA GCC AAG GAA GGC TCC GAG GCA GGG CCA GCT GCC ATC TCT ATC ATC GCC CAG
 481 K G T A K E G S E A G P A A I S I I A Q
 511
 GCT GAG TGT GAG AAT AGC CAA GAG TTC AGC CCC AGC TTT TCA GAA CGC ATT TTC ATC GCT
 541 A E C E N S Q E F S P T F S E R I F I A
 571
 GGG TCC AAA CAG TAC AGC CAG TCC GAG AGT CTT GAT CAG ATC CCC AAC AAT GTG GCC CAT
 G S K Q Y S Q S E S L D Q I P N N V A H

FIG. 6A

13/46

601 GCT ACA GAG GGC AAA ATG GCC CGT GTG TGT TGG AAG GGA AAG CGT CGC AGC AAA GCC GCG
 A T E G K M A R V C 631
 661 AAG AAA CGG AAG AAG AAG AGC TCA AAG TCC CTG GCT CAT GCA GGA GTG GCC ITG GCC AAA
 K K R K K K S S K S L A H A G A L A K 691
 721 CCC CTC CCC AGC CCT CAG GAG AGC TGC ACC ATC CCA GTG CAG GAT GAT TCT
 P L P R T P E Q E S C T I P V Q E D E S 751
 781 CCA CTC GGC GCC CCA TAT GTT AGA AAC ACC CCG CAG TTC ACC AAG CCT CTG AAG GAA CCA
 P L G A P Y V R N T P Q F T K P L K E P 811
 841 GGC CTT GGG CAA CTC TGT TTT AAG CAG CTT GGC GAG GGC CTA CGG CGG GCT CTG CCT CGA
 G L G Q L C F K Q L G E G L R P A L P R 871
 901 TCA GAA CTC CAC AAA CTG ATC AGC CGC TTG CAA TGT CTG AAC CAC CTG TGG AAA CTG CAC
 S E L H K L I S P L Q C L N H V W K L H 931
 961 CCC CAG GAC GGA GGC CCC CTG CCC CTG CCC AGC CAC CCC TTC CCC TAT AGC AGA CTG
 H P Q D G G P L P L P T H P P F P Y S R L 991
 1021 CCT CAT CCC TTC CCA TTC CAC CCT CTC CAG CCC TGG AAA CCT CAC CCT CTG GAG TCC TTC
 P H P F P F H P L Q P W K P H P L E S F 1051
 1081 CTG GGC AAA CTG GCC TGT GTA GAC ACC CAG AAA CCC TTG CCT GAC CCA CAC CTG AGC AAA
 L G K L A C V D S Q K P L P D P H L S K 1111
 1141 CTG GCC TGT GTA GAC AGT CCA AAG CCC CTG CCC GGC CCA CAC CTG CAG CCC AGC TGC CTG
 L A C V D S P K P L P G P H L E P S C L 1171
 1201 TCT GGT GGT CAC CAT GAG AAG TTT TCT GTG GAG GAA TAC CTA GTG CAT GCT CTG CAA GGC
 S R G A H E K F S V E E Y L V H A L Q G 1231
 1261 AGC GTG AGC TCA AGC CAG GCC CAC AGC CTG ACC CAG CTG GCC AGC TGG GCA CCA R
 S V S S S Q A H S L T S L A K T W A A 1291

FIG. 6B

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1321 GGC TCC AGA TCC CGG GAG CCC AGC CCC AAA GAT GAG GAC AAC GAG GGT GTC CTG CTC ACT
 G S R S R E P S P K T E D N E G V L L T
 1381 GAG AAA CTC AAG CCA GTG GAT TAT GAG TAC CGA GAA GAA GTC CAC TGG GCC ACG CAC CAG
 E K L K P V D Y E Y R E V H W A T H Q
 1441 CTC CGC CTG GGC AGA GGC TCC TTC GGA GAG GTG CAC AGG ATG GAG GAC AAC CAG ACT GGC
 L R L G R G S F G E V H R M E D K Q T G
 1501 TTC CAG TGC GCT GTC AAA AAG GTG GGC CTG GAA GTA TTT CGG GCA GAG GAG CTG ATG GCA
 F Q C A V K K V R L E V F R A E E L M A
 1561 TGT GCA GGA TTG ACC TCA CCC AGA ATT GTC CCT TTG TAT GGA GCT GTG AGA GAA GGG CCT
 C A G L T S P R I V P L Y G A V R E G P
 1621 TGG CTC AAC ATC TTC ATG GAG CTG GAA GGT GGC TCC CTG GGC CAG CTG CTC AAG GAG
 M V N I F M E L L E G G S L G Q L V K E
 1681 CAG GGC TGT CTC CCA GAG GAC CGG GCC CTG TAC TAC CTG GGC CAG GCC CTG GAG GGT CTG
 Q G C L P E D R A L Y Y L G Q A L E G L
 1741 GAA TAC CTC CAC TCA CGA AGG ATT CTG CAT GGG GAC GTC AAA GCT GAC AAC GTG CTC CTG
 E Y L H S R R I L H G D V K A D N V L L
 1801 TCC AGC GAT GGG AGC CAC GCA GCC CTC TGT GAC TTT GGC CAT GCT GTG TGT CTT CAA CCT
 S S D G S H A A L C D F G H A V C L Q P
 1861 GAT GGC CTG GGA AAG TCC TTG CTC ACA GGG GAC TAC ATC CCT GGC ACA GAG ACC CAC ATG
 D G L G K S L L T G D Y I P G T E T H M
 1921 GCT CCG GAG GTG GTG CTG GGC AGG AGC TGC GAC GCC AAG GTG GAT GTC TGG AGC AGC TGC
 A P E V V L G R S C D A K V D V W S S C

FIG. 6C

1981 TGT ATG ATG CTG CAC ATG CTC AAC GGC TGC CAC CCC TGG ACT CAG TTC CAG TTT GGA GGG CCG
 C M H L H M L N G C H P 2011
 2041 CTC TGC CTC AAG ATT GCC AGC GAG CTT CCG CTT GTG AGG GAG ATC CCA CCC TCC TGC GCC
 L C L K I A S E P P P V R E I P P S C A 2071
 2101 CCT CTC ACA GCC CAG GCC ATC CAA GAG GGG CTC AGG AAA GAG CCC ATC CAC GGC GTG TCT
 P L T A Q A I Q E G L R K E P I H R V S 2131
 2161 GCA GAG CTG CAG GGG AAG GTG AAC CGG GCA CTA CAG CAA GTG GGA GGT CTG AAG AGC
 A A E L G G K V N R A L Q Q V G L K K S 2191
 2221 CCT TGG AGG GGA GAA TAT AAA GAA CCA AGA CAT CCA CGG CCA AAT CAA GGC AAT TAC CAC
 P W R G E Y K E P R H P P P N Q A N Y H 2251
 2281 CAG ACC CTC CAT GCC CAG CGG AGA GAG CTT TGG CCA AGG GCC CCA GGG CCC GGG CCA GCT
 Q T L H A Q P R E L S P R A P G P R P A 2311
 2341 GAG GAG ACA ACA GGC AGA GCC CCT AAG CTC CAG CCT CCT CTC CCA CCA GAG CCC CCA GAG
 E T I G R A P K L Q P P L P P E P P E 2371
 2401 CCA AAG AAG TCT CCT CCC TTG ACT TTG AGC AAG GAG GAG TCT GGG ATG TGG GAA CCC TTA
 P N K S P P L T L S K E S G M W E P L 2431
 2461 CCT CTG TCC TCC CTG CAG CCA GGC CCT GCC AGA AAC CCC AGC TCA CCA GAG CGG AAA GCA
 P L S S L E P A P A R N P S S P E R K A 2491
 2521 ACC GTC CCG GAG CAG GAA CTG CAG CAG CTG GAA ATA GAA TTA TTC CTC AAC AGC CTG TCC
 T V P E Q E L Q Q L E I E L F L N S L S 2551
 2581 CAG CCA TTT TCT CTG GAG GAG CAG CAA ATT CTC TGC TGC CTC AGC ATC GAC AGC CTC
 Q P F S L E E Q E Q I L S C L S I D S L 2611
 2641 TCC CTG TCG GAT GAC AGT GAG AAG AAC CCA TCA AAG GCC TCT CAA AGC TCG CGG GNC ACC
 S L S D S E K N P S K A S Q S S R D T 2671
 2701 CTG AGC TCA GCC GTA CAC TCC TGG AGC AGC CAG GCC CAG GCT CGA AGC TCC AGC TGG AAG
 L S S G V H S W S S Q A E A R S S W N 2731
 2761 ATG GTC CTG GCC CGG GGG CCC ACC CAC ACC CCA AGC TAT TTC AAT GGT GTG AAA GTC
 M V L A R G R P T D T P S Y F N G V K V 2791

FIG. 6D

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2821 CNA ATA CAG TCT CTT AAT GGT GAA CAC CTG CAC ATC CCG CAG TTC CAC CCG GTC AAA GTG
 Q I Q S L N G E H L H I R E F H R V K V
 2881 GGA GAC ATC GCC ACT GGC ATC AGC CAG ATC CCA GCT GCA GCC TTC AGC TTG GTC ACC
 G D I A T G I S S Q I P A A F S L V T
 2941 AAA GAC GGG CAG CCT GTT CGC TAC GAC ATG GAG GTG CCA GAC TCG GGC ATC GAC CTG CAG
 K D G Q P V R Y D M E V P D S G I D L Q
 3001 TGC ACA CTG GCC CCT GAT GGC AGC TTC GCC TGG AGC TGG AGG GTC AAG CAT GGC CAG CTG
 C T L A P D G S F A W S W R V K H G Q L
 3061 GAG AAC AGG OCC TAA CCC TGC CCT CCA CCG CCG GCT CCA CAC TGC CCG AAA GCA GGC TTC
 E N R P *
 3121 CTG CTC GGT GCA CGA TGC TGC CCT GAA AAC ACA GGC TCA GCC GTT CCC AGG GGA TTG CCA
 3181 GCC CCC CCG CTC ACA GTG GGA ACC AGG GCC TCG CAG CAG CAA GGT GGG GGC AAG CAG AAT
 3241 GCC TCC CAG GAT TTC ACA CCT CAG CCC TGC CCC ACC CTG CTG AAA AAA CAT CCG CCA CGT
 3301 GAA GAG ACA GAA GGA TGG CAG GAG TTA CCT GGG GAA ACA AAA CAG GGA TCT TTT TCT
 3361 GCC CCT GCT CCA GTC GAG TTG GCC TGA CCC GCT TGG ATC AGT GAC CAT TTG TTG GCA GAC
 3421 AGG GGA GAG CAG CTT CCA GCC TGG GTC ACA AGG GGT GGG CGA GCC CTT CCG CCC CTC ACC

Fig. 6E

3481 CTC CAG GCT GCT GTG AGA GTG TCA AGT GTG TAA GGG CCC AAA CTC AGG TTC ACT GCA GAA 3511
 3541 CCA GGT CAG CAG GTA TGC CCG CCC GTA GGT TAA GGG GGC CCT CTA AAC CCC TTG CCT GGC 3571
 3601 CTC ACC TGG CCA GCT CAC CCC TTT TGG GTG TAG GGG AAA AGA ATG CCT GAC CCT GGG AAG 3631
 3661 GCT CCC TGG TAG AAT ACA CCA CAC TTT TCA GGT TGT TGC AAC ACA GGT CCT GAG TTG ACC 3691
 3721 TCT GGT TCA GCC AAG GAC CAA AGA AGG TGT GTA AGT GAA GTG GTT CTC AGT CCC CAG ACA 3751
 3781 TGT GCC CCT TTG CTG CTG GCT ACC ACT CTT CCC CAG AGC AGC AGG CCC CGA GCC CCT TCA 3811
 3841 GGC CCA GCA CTG CCC CAG ACT CGC TGG CAC TCA GTT CCC TCA TCT GTA AAG GTG AAG GGT 3871
 3901 GAT GCA GGA TAT GCC TGA CAG GAA CAG TCT GTG GAT GGA CAT GAT CAG TGC TAA GGA AAG 3931
 3961 CAG CAG AGA GAG ACG TCC GGC GCC CCA GCC CCA CTA TCA GTG TCC AGC GTG CTG GTT CCC 3991
 4021 CAG AGC AGA GGT CAG CAT CAC ACT GAC ACT CAC CCT GCC CTG CCC CTG GGC AGA GGG TAC 4051
 4081 TGC CGA CGG CAC TTT GCA CTC TGA TGA CCT CAA AGC ACT TTC ATG GCT GCC CTC TGG CAG 4111

FIG. 6F

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4141 GGC AGG GCA GGG CAG TGA CAC TGT AGG AGC ATA GCA AGC CAG GAG ATG GGG TGA AGG GAC 4171
 4201 ACA GTC TTG AGC TGT CCA CAT GCA TGT GAC TCC TCA AAC CTC TTC CAG ATT TCT CTA AGA 4231
 4261 ATA GCA CCC CCT TCC CCA TTG CCC CAG CTT AGC CTC TTC CAG GGG AGC TAC TCA GGA 4291
 4321 CTC AGG TAG CAT TAA ATC AGC TGT GAA TCG TCA GGG GGT GTC TGC TAG CCT CAA CCT CCT 4351
 4381 GGG GCA GGG GAC GCC GAG ACT CCG TGG GAG AAG CTC ATT CCC ACA TCT TGC CAA GAC AGC 4411
 4441 CTT TGT CCA GGT GTC CAC ATT GNG TCA GAC TGC TCC CGG GGA GAG AGC CCC GGC CCC CAG 4471
 4501 CAC ATA AAG AAC TGC AGC CTT GGT ACT GCA GAG TCT GGG TTG TAG AGA ACT CTT TGT AAG 4531
 4561 CAA TAA AGT TTG GGG TGA TGA CAA ATG TTA AAA AAA 4596

FIG. 6G

[illegible]

SUBSTITUTE SHEET (RULE 26)

Year	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021	2022	2023	2024	2025	2026	2027	2028	2029	2030	2031	2032	2033	2034	2035	2036	2037	2038	2039	2040	2041	2042	2043	2044	2045	2046	2047	2048	2049	2050	2051	2052	2053	2054	2055	2056	2057	2058	2059	2060	2061	2062	2063	2064	2065	2066	2067	2068	2069	2070	2071	2072	2073	2074	2075	2076	2077	2078	2079	2080	2081	2082	2083	2084	2085	2086	2087	2088	2089	2090	2091	2092	2093	2094	2095	2096	2097	2098	2099	2100
1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021	2022	2023	2024	2025	2026	2027	2028	2029	2030	2031	2032	2033	2034	2035	2036	2037	2038	2039	2040	2041	2042	2043	2044	2045	2046	2047	2048	2049	2050	2051	2052	2053	2054	2055	2056	2057	2058	2059	2060	2061	2062	2063	2064	2065	2066	2067	2068	2069	2070	2071	2072	2073	2074	2075	2076	2077	2078	2079	2080	2081	2082	2083	2084	2085	2086	2087	2088	2089	2090	2091	2092	2093	2094	2095	2096	2097	2098	2099	2100	

FIG. 7B

s3	1	-	-	-	-	0	-	-	-	0
s4	1	-	-	-	-	0	-	-	-	0
s5	1	-	-	-	-	0	-	-	-	0
s6	1	-	-	-	-	0	-	-	-	0
s7	1	-	-	-	-	0	-	-	-	0
s8	1	-	-	-	-	0	-	-	-	0
s9	1	-	-	-	-	0	-	-	-	0
s10	1	-	-	-	-	0	-	-	-	0
s11	1	-	-	-	-	0	-	-	-	0
s12	1	-	-	-	-	0	-	-	-	0
s13	1	-	-	-	-	0	-	-	-	0
s14	1	-	-	-	-	0	-	-	-	0
s15	1	-	-	-	-	0	-	-	-	0
s16	1	-	-	-	-	0	-	-	-	0
s17	1	-	-	-	-	0	-	-	-	0
s18	1	-	-	-	-	0	-	-	-	0
s19	1	-	-	-	-	0	-	-	-	0
s20	1	-	-	-	-	0	-	-	-	0
s21	1	-	-	-	-	0	-	-	-	0
s22	1	-	-	-	-	0	-	-	-	0
s23	1	-	-	-	-	0	-	-	-	0
s24	1	-	-	-	-	0	-	-	-	0
s25	1	-	-	-	-	0	-	-	-	0
s26	1	-	-	-	-	0	-	-	-	0
s27	1	-	-	-	-	0	-	-	-	0
s28	1	-	-	-	-	0	-	-	-	0
s29	1	-	-	-	-	0	-	-	-	0
s30	1	-	-	-	-	0	-	-	-	0
s31	1	-	-	-	-	0	-	-	-	0
s32	1	-	-	-	-	0	-	-	-	0
s33	1	-	-	-	-	0	-	-	-	0
s34	1	-	-	-	-	0	-	-	-	0
s35	1	-	-	-	-	0	-	-	-	0
s36	1	-	-	-	-	0	-	-	-	0
s37	1	-	-	-	-	0	-	-	-	0
s38	1	-	-	-	-	0	-	-	-	0
s39	1	-	-	-	-	0	-	-	-	0
s40	1	-	-	-	-	0	-	-	-	0
s41	1	-	-	-	-	0	-	-	-	0
s42	1	-	-	-	-	0	-	-	-	0
s43	1	-	-	-	-	0	-	-	-	0
s44	1	-	-	-	-	0	-	-	-	0
s45	1	-	-	-	-	0	-	-	-	0
s46	1	-	-	-	-	0	-	-	-	0
s47	1	-	-	-	-	0	-	-	-	0
s48	1	-	-	-	-	0	-	-	-	0
s49	1	-	-	-	-	0	-	-	-	0
s50	1	-	-	-	-	0	-	-	-	0
s51	1	-	-	-	-	0	-	-	-	0
s52	1	-	-	-	-	0	-	-	-	0
s53	1	-	-	-	-	0	-	-	-	0
s54	1	-	-	-	-	0	-	-	-	0
s55	1	-	-	-	-	0	-	-	-	0
s56	1	-	-	-	-	0	-	-	-	0
s57	1	-	-	-	-	0	-	-	-	0
s58	1	-	-	-	-	0	-	-	-	0
s59	1	-	-	-	-	0	-	-	-	0
s60	1	-	-	-	-	0	-	-	-	0
s61	1	-	-	-	-	0	-	-	-	0
s62	1	-	-	-	-	0	-	-	-	0
s63	1	-	-	-	-	0	-	-	-	0
s64	1	-	-	-	-	0	-	-	-	0
s65	1	-	-	-	-	0	-	-	-	0
s66	1	-	-	-	-	0	-	-	-	0
s6										

FIG. 7C

[illegible]

SUBSTITUTE SHEET (RULE 26)

s3	1	-	-	-	-	0	-	-	0																										
s4	1	-	-	-	-	-	-	-	0																										
s9	1	-	-	-	-	0	-	-	0																										
s7	1	-	-	-	-	0	-	-	0																										
s2	1	-	-	-	-	-	-	-	0																										
s6	1	-	-	-	-	-	-	-	0																										
s8	265	L	S	P	T	K	S	G	P	S	K	D	E	K	N	F	L	H	S	T	S	T	H	Q	K	T	K	S	A	S	S	L	Y	297	
s1	1	-	-	-	-	-	-	-	-	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0		
s5	159	L	A	R	V	L	K	G	D	I	V	K	G	E	K	T	R	I	A	N	Q	V	K	K	P	G	L	N	K	E	L	S	D	E	191
s3	1	-	-	-	-	-	-	-	-	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0		
s4	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0		
s9	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0		
s7	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0		
s2	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0		
s6	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0		
s8	298	R	R	S	F	I	S	L	R	G	S	S	S	S	N	A	S	S	A	K	S	P	N	I	K	S	I	P	A	R	P	H	330		
s1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0		
s5	192	I	W	L	E	L	K	A	W	L	N	G	R	T	M	Q	E	M	E	Q	S	L	T	Y	L	R	D	S	S	D	S	V	F	E	224

FIG. 7E

SUBSTITUTE SHEET (RULE 26)

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s3 1 - - - - - 0 - - - - -
 s4 1 - - - - - 0 - - - - -
 s9 1 - - - - - 0 - - - - -
 s7 1 - - - - - 0 - - - - -
 s2 1 - - - - - 0 - - - - -
 s6 1 - - - - - 0 - - - - -
 s8 397 LAKGSKHYETNVSSPLKQSSLP TSDDKGNLWN 429
 s1 1 - - - - - 0 - - - - -
 s5 291 FKTNLTLRRQELDDWINRFSP ISSSDNCQEDFD 323

 s3 1 - - - - - 0 - - - - -
 s4 1 - - - - - 0 - - - - -
 s9 1 - - - - - 0 - - - - -
 s7 1 - - - - - 0 - - - - -
 s2 1 - - - - - 0 - - - - -
 s6 1 - - - - - 0 - - - - -
 s8 430 KFKRSQIGVPSNPTVAYVTSQETPSLKSNST 462
 s1 1 - - - - - 0 - - - - -
 s5 324 GVPQWNCKMKILAEQLMKEKNIESIFQK - - - - - 351

FIG. 7G

FIG. 7H

SUBSTITUTE SHEET (RULE 26)

[illegible]

[illegible]

```

1  - - - - - 0
s3
1  - - - - - 0
s4
1  - - - - - 0
s9
1  - - - - - 0
s7
1  - - - - - 0
s2
1  - - - - - MEY 4
s6
1  - - - - - MEQTQAEGTDL 14
s8
1  - - - - - NNQKYSVKNFLDQKFYPLKKTGLNDSNKYIL 561
529
1  - - - - - 0
s1
1  - - - - - YARKLKNPTMMIDQMDDFNAFIRLSVQLKYT 440
s5

1  - - - - - 0
s3
1  - - - - - 0
s4
1  - - - - - 0
s9
1  - - - - - 0
s7
1  - - - - - VAEWLKSIG-LEKYIEQFS 26
5
TSK - - - - - EKTNDLPFVQLFLEEIG-CTQYLDSSF 43
s2
GD - - - - - EKTNDLPFVQLFLEEIG-CTQYLDSSF 43
s6
15 VTK - - - - - NVSFVPLNLKSVAKLSSFKESAL 568
s8
562
1  - - - - - LTKYCSNLPFDVDFDPTFENTVIEAIRYLFFLL 473
s1
1  - - - - - 0
s4
441
s5

```

FIG. 7I

[illegible]

SUBSTITUTE SHEET (RULE 26)

[illegible]

SUBSTITUTE SHEET (RULE 26)

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s3 1 - - - - - 0
 s4 1 - - - - - 0
 s9 1 - - - - - 0
 s7 1 - - - - - 0
 s2 102 FSLEDASK - - - - - FIVGVSSSR - - IKLI 123
 s6 156 LLATNSNGEVT KMVDYDVFVLDYTKNVHL LLY 188
 s8 715 VSNTPTEELNYWNI KEVLSHEENAPKMVF KTS 747
 s1 13 VTMLNASGSTHFTRMRRRLMAIADEVEIAEVIQ 45
 s5 604 SGNTFESSGVYMF AAPEL LGCDNDTILRLRNK 636

 s3 1 - - - - - 0
 s4 1 - - - - - 0
 s9 1 - - - - - MAVMEMACPGAPG 13
 s7 1 - - - - - 0
 s2 124 TEEEFKQICFNS - - - - - SSPE RDRLIIVP 147
 s6 189 DVELVTICHAN - - - - - DRVEKNRLIFVSK 212
 s8 740 PKLELNI.PDKGSKLNIPTITENESKSSFQVLR 780

FIG. 7L

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FIG. 7M

[illegible][illegible]

FIG. 7N

[illegible]

FIG. 70

Year	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021	2022	2023	2024	2025	2026	2027	2028	2029	2030	2031	2032	2033	2034	2035	2036	2037	2038	2039	2040	2041	2042	2043	2044	2045	2046	2047	2048	2049	2050	2051	2052	2053	2054	2055	2056	2057	2058	2059	2060	2061	2062	2063	2064	2065	2066	2067	2068	2069	2070	2071	2072	2073	2074	2075	2076	2077	2078	2079	2080	2081	2082	2083	2084	2085	2086	2087	2088	2089	2090	2091	2092	2093	2094	2095	2096	2097	2098	2099	2100
1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021	2022	2023	2024	2025	2026	2027	2028	2029	2030	2031	2032	2033	2034	2035	2036	2037	2038	2039	2040	2041	2042	2043	2044	2045	2046	2047	2048	2049	2050	2051	2052	2053	2054	2055	2056	2057	2058	2059	2060	2061	2062	2063	2064	2065	2066	2067	2068	2069	2070	2071	2072	2073	2074	2075	2076	2077	2078	2079	2080	2081	2082	2083	2084	2085	2086	2087	2088	2089	2090	2091	2092	2093	2094	2095	2096	2097	2098	2099	2100	

[illegible]

FIG. 7P

s3	12	EIE D L I N H	N V S E V D I M E N T	V A S E E P A V	E E R
s4	12	E E E D L L K H I	N V S B V I O M E N T	V A S E E R A V	E E R
s9	278	H P L E S F G K L	A C V D S Q K P L	P D P H L S	K L A G A V D S R
s7	1	- - - - -	T I T H T S L U F	A P P N L S	P M Q D F I G S
s2	351	Q V I S - - - - -	- - - - -	F S R G S	S P S F I E Q R
s6	369	- - - - -	- - - - -	R P E D N	D A I S N O L E
s8	1037	Q N I E K F E P R A N I	D K P I T E G I A S	B I S P K S	D S I L L
s1	336	I C K K E K M E A E E E E	A L A A M A M S A S	Q D A L P	V A Q L
s5	932	E L K E R V - - - - -	- - - - -	S V O M S L	S H F
s3	45	S I M T M C P D S	N Q N K E H S E S I	L R S G Q E V	- - P W H S
s4	45	S I M T M C Q D S	N Q N D E R S K S I	L S G Q E V	- - P W H S
s9	311	K P L P G H L E P S C L S	R G A H E K F S	V E I Y L V H A I	Q Q C
s7	24	V R R S I I V F K Q S G D F	D T G A A G V G S	G F G G F V E K I	G S
s2	366	S P I S P S T I T S E D - - - - -	- - - - -	- - - - -	- - - - -
s6	392	S I S V E S G E E E - - - - -	- - - - -	- - - - -	- - - - -
s8	1070	S P K N V A S S R T E P S T P S	A P V P P D S	S S Y E F I Q D G	N I 102
s1	369	Q V E N G E D I I I Q Q D T I P	E T I P G H - - - - -	- - - - -	- - - - -
s5	949	D V M G A R A T E A E N G M Q Q A R	N I D I E E N I D E E E A	T I T I	981

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S3 75 S V R Y G T V E D I - L A F A N H S N T T K H E M R C I R P - - 103
 S4 75 S V R Y G T V E B I - L A F A N H S N T T A K H E M G Q I R P - - 103
 S9 344 S Y S S Q A H S I - T S L A K T W A A R G S R S R E P S B K T E - - 375
 S7 57 S I R K S S I G I S K A H V P A I P S I I S K A E I P A K A R K D 89
 S2 380 - 392
 S6 393 - 412
 S8 1103 G K N K P L N Q A K T P K R K T I R T A H E A S L A H K N S V 1135
 S1 391 - 402
 S5 982 E I I N S R I R L E A I K I E K T M K R N P R Q M G K M L D A T D 1014

 S3 104 Q E S G L L N M - - - - - V I S P Q N G R Y Q I D S D M V I V P 131
 S4 104 Q E S G L L N M - - - - - V I T P Q N G R Y Q I D S D M V I V P 131
 S9 376 D N E G V L L T E - - - - - K L K B V D - - - - - Y E Y R E E M H W A I 401
 S7 90 D T I P P I - 94
 S2 393 - - - - - K - 393
 S6 413 K N - 414
 S8 1136 K L K R O N T K M W G T R M V E T E N H M V S I N K A K N S K G 1188

FIG. 7R

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s3	132	W	K	L	T	Y	R	S	G	S	G	F	V	P	R	G	A	F	G	K	V	Y	A	C	D	M	K	T	K	R	M	164	
s4	132	W	K	L	T	Y	R	N	G	S	D	I	F	P	R	G	A	F	G	K	V	Y	A	C	D	M	K	T	K	R	M	164	
s9	402	H	Q	L	R	-	-	-	-	-	-	-	-	L	G	R	G	S	F	G	Q	V	Y	A	C	D	M	K	T	K	R	426	
s7	95	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	122		
s2	394	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	420		
s6	415	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	442		
s8	1169	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1201		
s1	403	E	Y	K	E	F	A	M	K	G	E	M	I	G	K	G	S	F	G	Q	V	Y	A	C	D	M	K	T	K	R	M	429	
s5	1028	I	S	N	V	S	M	R	W	Q	K	R	S	F	I	G	G	T	F	G	Q	V	Y	A	C	D	M	K	T	K	R	M	1060
s3	165	A	C	K	L	I	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	169	
s4	165	A	C	K	L	I	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	169	
s9	427	A	V	K	K	V	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	431	
s7	123	A	V	K	K	V	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	127	
s2	421	A	V	K	K	V	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	425	
s6	443	V	K	Q	V	E	I	K	N	N	I	G	V	P	T	D	N	K	Q	A	N	S	D	E	N	E	Q	E	Q	E	Q	475	
s8	1202	A	V	K	K	V	E	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1207	
s1	430	A	V	K	K	V	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	436	
s5	1061	A	V	K	K	V	I	K	I	H	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1068	

FIG. 75

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s3	170	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
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FIG. 7T

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SUBSTITUTE SHEET (RULE 26)

SUBSTITUTE SHEET (RULE 26)

[illegible]

FIG. 7X

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S3	418	E	L	P	E	N	A	D	S	S	O	G	S	T	E	S	E	N	L	R	C	R	S	V	D	L	G	A	450	
S4	418	E	L	P	E	N	A	D	S	S	O	G	S	T	E	S	E	N	L	R	C	R	S	V	D	L	G	A	450	
S9	720	E	P	E	P	N	K	S	P	P	L	T	S	K	E	S	G	M	E	P	L	P	S	S	L	E	P	A	752	
S7	425	E	S	L	W	K	L	G	N	S	O	D	D	M	C	Q	M	D	N	D	M	F	G	A	S	M	K	S	457	
S2	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	659			
S6	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	717			
S8	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1478			
S1	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	672			
S5	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1314			
S3	451	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	461		
S4	451	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	461		
S9	753	A	R	N	P	S	S	P	E	R	K	A	T	V	P	E	Q	E	L	Q	L	E	-	-	-	-	-	-	-	705
S7	458	H	S	P	A	N	Y	K	S	F	N	P	M	C	E	P	D	N	D	W	P	C	K	H	D	E	S	P	E	490
S2	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	659		
S6	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	717		
S8	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1478		
S1	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	672		
S5	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1314		

FIG. 7Y

FIG. 7Z

FIG. 7AA

[illegible]

SUBSTITUTE SHEET (RULE 26)

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:

(A) NAME: Yeda Research and Development Co. Ltd.
 (B) STREET: Weizmann Institute of Science
 (C) CITY: P.O.B. 95
 (D) STATE: Rehovot
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(A) NAME: David Wallach
 (B) STREET: 24 Borochoy Street
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(A) NAME: Nikolai Malinin
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 (F) POSTAL CODE (ZIP): 76100

(A) NAME: Mark Boldin
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 (E) COUNTRY: Israel
 (F) POSTAL CODE (ZIP): 76100

(A) NAME: Andrei Kovalenko
 (B) STREET: Beit Clore, Weizmann Institute of Science
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 (E) COUNTRY: Israel
 (F) POSTAL CODE (ZIP): 76100

(A) NAME: Igor Mett
 (B) STREET: 60 Levin Epstein Street
 (C) CITY: Rehovot
 (E) COUNTRY: Israel
 (F) POSTAL CODE (ZIP): 76462

(ii) TITLE OF INVENTION: Modulators of TNF Receptor Associated Factor (TRAF), their Preparation and Use

(iii) NUMBER OF SEQUENCES: 11

(iv) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk
 (B) COMPUTER: IBM PC compatible
 (C) OPERATING SYSTEM: PC-DOS/MS-DOS
 (D) SOFTWARE: PatentIn Release #1.0, Version #1.30 (EPO)

(v) CURRENT APPLICATION DATA:

APPLICATION NUMBER: PCT/IL97/00117

(vi) PRIOR APPLICATION DATA:

(A) APPLICATION NUMBER: IL 117800
 (B) FILING DATE: 02-APR-1996

(vi) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: IL 119133
(B) FILING DATE: 26-AUG-1996

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1906 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

CATTGGGTCA	CGCGGTGGCG	CGGCTCTAGA	ATAGTGGATC	CCCCGGGCTG	CAGGAATTCTG	60
ATTTCGAGGCC	ACGAAGGCCG	CGGCGCGCGG	GCANGCACCG	GCCCGGGGAN	AGGCNCCATG	120
AGCGGATCNC	NGAACNATGA	CAAAAGACAA	TTTCTGCTGG	AGCGACTGCT	GGATGCAGTG	180
AAACAGTGCC	AGATCCGCTT	TNGAGGAGA	AAGGAGATTG	CCTCGGATTC	CGACAGCAGG	240
GTCACCTGTC	TGTTGCCCCA	GTTTGAAGCC	GTCTGCAGC	ATGGCTTGAA	GAGGAGTCGA	300
GGATTGGCAC	TCACAGCGCG	AGCGATCAAG	CAGGCAGCGG	GCTTTGCCAG	CAAAACCGAA	360
ACAGAGCCCG	TGTTCTGGTA	CTACGTGAAG	GAGGTCCCTCA	ACAAGCACGA	GCTGCAGCGC	420
TTCTACTCCC	TGCGCCACAT	CGCCTCAGAC	GTGGGCCGGG	GTGCGCCTG	GCTGCGCTGT	480
GCCCTCAACG	AACACTCCCT	GGAGCGCTAC	CTGCACATGC	TCCTGGCCGA	CCGCTGCAGG	540
CTGAGCACTT	TTTATGAAGA	CTGGTCTTTT	GTGATGGATG	AAGAAAGGTC	CAGTATGCTT	600
CCTACCATGG	CAGCAGGTCT	GAACTCCATA	CTCTTTGCGA	TTAACATCGA	CAACAAGGAT	660
TGTAACGGGC	AGAGTAAGTT	TGCTCCACCC	GTTTCAGACC	TCTTAAAGGA	GTCAACGCAG	720
AACGTGACCT	CCTTGCTGAA	GGAGTCCACG	CAAGGAGTGA	GCAGCCTGTT	CAGGGAGATC	780
ACAGCCTCCT	CTGCGGTCTC	CATCCTCATC	AAACTGAAC	AGGAGACCGA	CCCTTGCTCG	840
TCGTGTCCAG	GAATGTCAGT	GCTGATGCCA	AATGCAAAAA	GGAGCGGAAG	AAGAAAAAGA	900
AAGTGACCAA	CATAATCTCA	TTTGATGATG	AGGAAGATGA	GCAGAACTCT	GGGACGTGT	960
TTAAAAAGAC	ACCTGGGGCA	GGGGAGAGCT	CAGAGGACAA	CTCCGACCCG	TCCTCTGTCA	1020
ATATCATGTC	CGCCTTTGAA	AGCCCTTCG	GGCCTAACTC	CAATGGAATC	AGAGCAGCAA	1080
CTCATGGAAA	ATTGATTCCC	TGTCCTTGAA	CGGGGAGTTT	GGGTACCAGA	AGCTTGATGT	1140
GAAAGCATC	GATGATGAAG	ATGTGGATGA	AAACGAAGAT	GACGTGTATG	GAAACTCATC	1200

AGGAAGGAAG CACAGGGGCC ACTCGGAGTC GCCCGAGAAG CCACTGGAAG GGAACACCTG 1260
 CCTCTCCCAG ATGCACAGCT GGGCTCCGCT GAAGTGCTG CACAATGACT CCGACATCCT 1320
 CTTCCCTGTC AGTGGCGTGG GCTCCTACAG CCCAGCAGAT GCGCCCCCTG GAAGCCTGGA 1380
 GAACGGGACA GGACCAGAGG ACCACGTTCT CCCGGATCCT GGACTTCGGT ACAGTGTTGA 1440
 AGCCAGCTCT CCAGGCCACG GAAGTCCTCT GAGCAGCCTG TTACTTCTGC CTCAGTGCCA 1500
 GAGTCCATGA CAATTAGTGA ACTGCGCCAG GCCACTGTGG CCATGATGAA CAGGAAGGAT 1560
 GAGCTGGAGG AGGAGAACAG ATCACTGCGA AACCTGCTCG ACGGTGAGAT GGAGCACTCA 1620
 GCCGCGCTCC GGCAAGAGGT GGACACCTTG AAAAGGAAGG TGGGTGAACA GGAGGAGCGG 1680
 CAGGGCATGA AGGTCCAGCG GCTGGCCAGC TATCTTTGCT ATTTTGTGAG GAGATTCTAA 1740
 CCCCACGTGA GAACCATGTG GTGGAGAAAT GGAGGGAGAG AGAAATCCAA CAGTTCCTGA 1800
 TAGTCTCATT TGAGCTCCTG GATCCAGTCT TTCTTGAAGC TGTGTTTCT CTGACTTTT 1860
 CATGTATGTG AGCCAATAAA TTGCTTTCAT TCCTTGAAAA AAAAAA 1906

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 604 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

Xaa Thr Gly Pro Gly Xaa Gly Xaa Met Ser Gly Ser Xaa Asn Xaa Asp
 1 5 10 15
 Lys Arg Gln Phe Leu Leu Glu Arg Leu Leu Asp Ala Val Lys Gln Cys
 20 25 30
 Gln Ile Arg Phe Xaa Gly Arg Lys Glu Ile Ala Ser Asp Ser Asp Ser
 35 40 45
 Arg Val Thr Cys Leu Cys Ala Gln Phe Glu Ala Val Leu Gln His Gly
 50 55 60
 Leu Lys Arg Ser Arg Gly Leu Ala Leu Thr Ala Ala Ala Ile Lys Gln
 65 70 75 80
 Ala Ala Gly Phe Ala Ser Lys Thr Glu Thr Glu Pro Val Phe Trp Tyr
 85 90
 Tyr Val Lys Glu Val Leu Asn Lys His Glu Leu Gln Arg Phe Tyr Ser
 100 105 110
 Leu Arg His Ile Ala Ser Asp Val Gly Arg Gly Arg Ala Trp Leu Arg

115					120					125					
Cys	Ala	Leu	Asn	Glu	His	Ser	Leu	Glu	Arg	Tyr	Leu	His	Met	Leu	Leu
130						135					140				
Ala	Asp	Arg	Cys	Arg	Leu	Ser	Thr	Phe	Tyr	Glu	Asp	Trp	Ser	Phe	Val
145					150					155					160
Met	Asp	Glu	Glu	Arg	Ser	Ser	Met	Leu	Pro	Thr	Met	Ala	Ala	Gly	Leu
				165					170					175	
Asn	Ser	Ile	Leu	Phe	Ala	Ile	Asn	Ile	Asp	Asn	Lys	Asp	Leu	Asn	Gly
		180						185					190		
Gln	Ser	Lys	Phe	Ala	Pro	Thr	Val	Ser	Asp	Leu	Leu	Lys	Glu	Ser	Thr
		195					200					205			
Gln	Asn	Val	Thr	Ser	Leu	Leu	Lys	Glu	Ser	Thr	Gln	Gly	Val	Ser	Ser
		210				215					220				
Leu	Phe	Arg	Glu	Ile	Thr	Ala	Ser	Ser	Ala	Val	Ser	Ile	Leu	Ile	Lys
225					230					235					240
Pro	Glu	Gln	Glu	Thr	Asp	Pro	Cys	Leu	Ser	Cys	Pro	Gly	Met	Ser	Val
				245					250					255	
Leu	Met	Pro	Asn	Ala	Lys	Arg	Ser	Gly	Arg	Arg	Lys	Arg	Lys	Xaa	Pro
			260					265					270		
Thr	Xaa	Ser	His	Leu	Met	Met	Arg	Lys	Met	Ser	Arg	Thr	Leu	Gly	Thr
		275					280					285			
Cys	Leu	Lys	Arg	His	Leu	Gly	Gln	Gly	Arg	Ala	Gln	Arg	Thr	Thr	Pro
		290				295					300				
Thr	Ala	Pro	Leu	Ser	Ile	Ser	Cys	Pro	Pro	Leu	Lys	Ala	Pro	Ser	Gly
305					310					315					320
Leu	Thr	Pro	Met	Glu	Ser	Glu	Gln	Gln	Leu	Met	Glu	Asn	Xaa	Phe	Pro
				325					330					335	
Val	Phe	Glu	Arg	Gly	Val	Trp	Val	Pro	Glu	Ala	Xaa	Cys	Glu	Lys	His
			340					345					350		
Arg	Xaa	Xaa	Arg	Cys	Gly	Xaa	Lys	Arg	Arg	Xaa	Arg	Val	Trp	Lys	Leu
		355					360					365			
Ile	Arg	Lys	Glu	Ala	Gln	Gly	Pro	Leu	Gly	Val	Ala	Arg	Glu	Ala	Thr
		370				375					380				
Gly	Arg	Glu	His	Leu	Pro	Leu	Pro	Asp	Ala	Gln	Leu	Gly	Ser	Ala	Glu
385					390					395					400
Gly	Ala	Ala	Gln	Xaa	Leu	Arg	His	Pro	Leu	Pro	Cys	Gln	Trp	Arg	Gly
				405					410					415	
Leu	Leu	Gln	Pro	Ser	Arg	Cys	Pro	Pro	Arg	Lys	Pro	Gly	Glu	Arg	Asp
			420						425				430		
Arg	Thr	Arg	Gly	Pro	Arg	Ser	Pro	Gly	Ser	Trp	Thr	Ser	Val	Gln	Cys
			435				440						445		

Gly Ser Gln Leu Ser Arg Pro Arg Lys Ser Ser Glu Gln Pro Val Thr
 450 455 460
 Ser Ala Ser Val Pro Glu Ser Met Thr Ile Ser Glu Leu Arg Gln Ala
 465 470 475 480
 Thr Val Ala Met Met Asn Arg Lys Asp Glu Leu Glu Glu Asn Arg
 485 490 495
 Ser Leu Arg Asn Leu Leu Asp Gly Glu Met Glu His Ser Ala Ala Leu
 500 505 510
 Arg Gln Glu Val Asp Thr Leu Lys Arg Lys Val Ala Glu Gln Glu Glu
 515 520 525
 Arg Gln Gly Met Lys Val Gln Ala Leu Ala Ser Tyr Leu Cys Tyr Phe
 530 535 540
 Val Arg Arg Phe Xaa Pro His Val Arg Thr Met Trp Trp Arg Asn Gly
 545 550 555 560
 Gly Arg Glu Lys Ser Asn Ser Ser Xaa Xaa Ser His Leu Ser Ser Trp
 565 570 575
 Ile Gln Ser Phe Leu Lys Leu Cys Phe Leu Trp Thr Phe His Val Cys
 580 585 590
 Glu Pro Ile Asn Cys Phe His Ser Leu Lys Lys Lys
 595 600

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 2631 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

CCCCCTCTCAC AGCCCCAGGCC ATCCAAGAGG GGCTGAGGAA AGAGCCCATC CACCGCGTGT	60
CTGCAGCGGA GCTGGGAGGS AAGGTGAACC GGGCACTACA GCAAGTGGA GGTCTGAAGA	120
GCCCTTGAG GGGAGATAT AAAGAACCAA GACATCCACC GCCAATCAA GCCAATTACC	180
ACCAGACCCT CCATGCCAG CCGAGAGAGC TTTCGCCAAG GGCCCCAGGG CCCCGGCCAG	240
CTGAGGAGAC AACAGGCAGA GCCCCTAAGC TCCAGCCTCC TCTCCACCA GAGCCCCAG	300
AGCCAAACAA GTCTCCTCCC TTGACTTTGA GCAAGGAGGA GTCTGGGATG TGGGAACCCCT	360
TACCTCTGTC CTCCCTGGAG CCAGCCCCTG CCAGAAACCC CAGCTCACCA GAGCGGAAG	420
CAACCGTCCC GGAGCAGGAA CTGCAGCAGC TGGAAATAGA ATTATCTCTC AACAGCCTGT	480

CCCAGCCATT	TTCTCTGGAG	GAGCAGGAGC	AAATTCTCTC	GTGCCTCAGC	ATCGACAGCC	540
TCTCCCTGTG	GGATGACAGT	GAGAAGAACC	CATCAAAGGC	CTCTCAAAGC	TCGCGGGACA	600
CCCTGAGCTC	AGGCGTACAC	TCCTGGAGCA	GCCAGGCCGA	GGCTCGAAGC	TCCAGCTGGA	660
ACATGGTGCT	GGCCCGGGGG	CGGCCACCCG	ACACCCCAAG	CTATTTCAT	GGTGTGAAAG	720
TCCAATACA	GTCTCTTAAT	GGTGAACACC	TGCACATCCG	GGAGTTCAC	CGGGTCAAAG	780
TGGGAGACAT	CGCCACTGGC	ATCAGCAGCC	AGATCCCAGC	TGCAGCCTTC	AGCTTGGTCA	840
CCAAAGACGG	GCAGCCTGTT	CGCTACGACA	TGGAGGTGCC	AGACTCGGGC	ATCGACCTGC	900
AGTGCACACT	GGCCCCGTAT	GGCAGCTTCG	CCTGGAGCTG	GAGGGTCAAG	CATGCCAGC	960
TGGAGAACAG	GCCCTAACCC	TGCCCTCCAC	CGCCGGCTCC	ACACTGCCGG	AAAGCAGCCT	1020
TCCTGCTCGG	TGCACGATGC	TGCCCTGAAA	ACACAGGCTC	AGCCGTTCCC	AGGGGATYTG	1080
NCCAGCCCCC	CGGCTCARCA	GNTGGGAACC	AGGGCCTCGN	CAGCNAGCNA	AGGTNGGGGG	1140
CAAGCNAGAA	TGCTCTCCAG	GATTTCACAN	CCTGAGCCCN	TGCCCCANCC	CTGCTGAADA	1200
AAACAYTNCC	GCCACGTGAA	GAGACAGAAG	GAGGATGGNC	AGGAGTTNNA	CCTYGGGGAA	1260
ACAAAACAGG	GATCTTTNTT	CTGCCCTGCG	TCCAGTNGCA	GTTGGCCTGN	ACCCGCTTGG	1320
ANTCAGTGAC	CATTTGTGG	CAGANCAAGG	GAGAGCAGCT	TCCAGCCTGG	GTGAGAAGGG	1380
GTGGGCGAGC	CCTTCGSCCC	CTACCCCTNC	CAGGCTGCTG	TGNAGATGT	CAAGTGTGTA	1440
AGGGNCCCAA	ANCTCAGGNT	TCAGTGCAGA	ACCAGGTNCA	GCAGGTATGC	CCGCCCCGNTA	1500
GGTTAANNNG	GGGCCCCCTN	AAACCCCTTG	CCTNNGGCTN	CACCTNNGCC	AGCTCANCCC	1560
CTTTTGGGTG	TAGGGGAAAA	GAATGCCTGA	CCCTGGGAAG	GCTWCCCTGG	TAGAATACAC	1620
CACACTTTTC	AGGTTGTTGC	AACACAGGTC	CTGAGTTGAC	CTCTGGTTCA	GCCAAGGACC	1680
AAAGAAGGTG	TGTAAGTGAA	GTGGTTCTCA	GTNCCCCAGA	CATGTGCCCC	TTTGCTGCTG	1740
GCTACCACTC	TTCCCCAGAG	CAGCAGGCCC	CGAGCCCCTT	CAGGCCAGC	ACTGCCCCAG	1800
ACTCGCTGGC	ACTCAGTTCC	CTCATCTGTA	AAGGTGAAGG	GTGATGCAGG	ATATGCCTGA	1860
CAGGAACAGT	CTGTGGATGG	ACATGATCAG	TGCTNAAGGN	AAAGCAGCAG	AGAGAGACGY	1920
TCCGGCGCCC	CAGNCCCCAC	TNATCAGTGT	NCCAGCGTGC	TNNGTTNCCC	CAGNAGACA	1980
GCTNCAGNCA	TCANCACTGA	CACTNCACCC	TNGCCCTGCC	CCTNNGCCAN	GAGGGTACTG	2040
CCGNACGGCA	CTTTGCACNT	CTGATGNACC	TCAAAGCACT	TTCATGGCTN	GCCCTCTNNG	2100
GCAGGGNCAG	GGNCGGGGNC	AGTGACANCT	GTAGGNAGCA	TANGCAANGC	CAGGAGATGG	2160
GGTGNAAGGG	ANCACTGTCT	TGAGCTGTCC	ANCATGCATG	TGACTNCCCT	AAACCTCTTN	2220
NCCAGNATTT	CTCTAAGAAT	AGCANCCCCC	TTNCCCCATT	GCCCCAGCTT	AGCCTCTTCT	2280

CCCAGGGGAG CTANCTCAGG ACTCACGTAG CATTAAATCA GCTGTGNAAT CGTCAGGGGG	2340
TGTCTGCTAG CCTCAACCTC CTGGGGCAGG GGACGCCGAG ACTCCGTGGG AGAAGCTCAT	2400
TCCCACATCT TGCCAAGACA GCCTTTNGTC CAGCTGTCCA CATTGAGTCA GACTGCTCCC	2460
GGGGAGAGAG CCCCGGCCCC CAGCACATAA AGAACTGCAG CCTTGGTACT GCAGAGTCTG	2520
GTTTGTAGAG AACTCTTTGT AAGCAATAAA GTTTGGGGTG ATGACAAATG TTAATAAAAG	2580
GCCTTCGTGG CCTCGAATCA AGCTTATCGA TACCGTCGAC CTCGAGGGGG G	2631

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1253 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

CATTGGAGTC ACGCGGTGCG GCGCTCTAG AATAGTGGAT CCCCGGGCTG CANGGAATTC	60
GATTTCGAGCC CACGAAGGCC CCTTCTTCTG TGGTGCGGGC ACGTTTACAG CCGCAAGCAC	120
CCAGCGGCAG CTGAAGGAGG CTTTGTGAGG GCTCCTGCCC CAGGTGGAGG CGGCCCCGAA	180
GGCCATCCGC GCCGCTCAGG TGGAGCGCTA TGTGCCCGAA CACGAGCGAT GCTGCTGGTG	240
CCTGTGCTGC GGCTGTGAGG TCGGGGAACA CCTGAGCCAT GGAACCTGA CGGTGCTGTA	300
CGGGGGGCTG CTGGAGCATC TGGCCAGCCC AGAGCACAAG AAAGCAACCA ACAATTCTG	360
GTGGGAGAAC AAAGCTGAGG TCCAGATGAA AGAGAAGTTT CTGGTCACTC CCCAGGATTA	420
TGCGCGATT C AAGAAATCCA TGGTGAAAGG TTTGGATTCC TATGAAGAAA AGGAGGATAA	480
AGTGATCAAG GAGATGGCAG CTCAGATCCG TGAGGTGGAG CAGAGCCGAC AGGAGGTGGT	540
TGGTCTGTG TTAGAGCCTC AGGCAGTGCC AGACCCAGAA GAGGGCTCTT CAGCACCTAG	600
AAGCTGGAAG GGGATGAACA GCCAAGTAGC TTCCAGCTTA CAGCAGCCCT CAAATTGGA	660
CCTGCCACCA GCTCCAGAGC TTGACTGGAT GGAGACAGGA CCATCTCTGA CATTCAATTGG	720
CCATCAGGAT ATACCAGGAG TTGGTAACAT CCACTCAGGT GCCACACCTC CCTGGATGAT	780
CCAAGATGAA GAATACATTG CTGGGAACCA AGAAATAGGA CCATCTCTAT AAGAAATTTCT	840
TAAAGAAAAG GAAAAACAGA AGTTGAAAAA ACTCCCCCA GACCGAGTTG GGGCCAACTT	900
TGATCACAGC TCCAGGACCA GTGCAGGCTG GCTGCCCTCT TTTGGGCCGC GTCTGGAATA	960
ATGGAGCCCG CTGGCAGTCC AGACATCAAC TCCAAACTG AAGCTGCAGC AATGAAGAAG	1020

CAGTCACATA CAGAAAAAAG CTAATCATGC TCTCTACCAA CTACCATGAG GCTAAAAGCC	1080
AAAGTCAACC AAACCCCTAT TATACCTTCC ACCCAAATTC TTTATCATTG TCTTCTTAG	1140
GAAACAGACA TACTATTCA TTTGATTAA TAAAGTTTA TTTTTCGGCC TTCGTGGCCT	1200
CGAATCAAGC TTATCGATAC CGTCGACCTC GAGGGGGGGC CGTACCCACT TTT	1253

(2) INFORMATION FOR SEQ ID NO: 5:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 417 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

Ile	Gly	Val	Thr	Arg	Trp	Arg	Arg	Ser	Arg	Ile	Val	Asp	Pro	Arg	Ala
1				5				10						15	
Ala	Xaa	Asn	Ser	Ile	Arg	Ala	His	Glu	Gly	Pro	Phe	Phe	Cys	Gly	Arg
		20						25					30		
Gly	Thr	Phe	Thr	Ala	Ala	Ser	Thr	Gln	Arg	Gln	Leu	Lys	Glu	Ala	Phe
		35					40					45			
Glu	Arg	Leu	Leu	Pro	Gln	Val	Glu	Ala	Ala	Arg	Lys	Ala	Ile	Arg	Ala
		50			55					60					
Ala	Gln	Val	Glu	Arg	Tyr	Val	Pro	Glu	His	Glu	Arg	Cys	Cys	Trp	Cys
65				70						75				80	
Leu	Cys	Cys	Gly	Cys	Glu	Val	Arg	Glu	His	Leu	Ser	His	Gly	Asn	Leu
			85					90						95	
Thr	Val	Leu	Tyr	Gly	Gly	Leu	Leu	Glu	His	Leu	Ala	Ser	Pro	Glu	His
		100						105						110	
Lys	Lys	Ala	Thr	Asn	Lys	Phe	Trp	Trp	Glu	Asn	Lys	Ala	Glu	Val	Gln
		115					120					125			
Met	Lys	Glu	Lys	Phe	Leu	Val	Thr	Pro	Gln	Asp	Tyr	Ala	Arg	Phe	Lys
		130					135					140			
Lys	Ser	Met	Val	Lys	Gly	Leu	Asp	Ser	Tyr	Glu	Glu	Lys	Glu	Asp	Lys
145				150						155				160	
Val	Ile	Lys	Glu	Met	Ala	Ala	Gln	Ile	Arg	Glu	Val	Glu	Gln	Ser	Arg
			165					170						175	
Gln	Glu	Val	Val	Arg	Ser	Val	Leu	Glu	Pro	Gln	Ala	Val	Pro	Asp	Pro
			180					185					190		
Glu	Glu	Gly	Ser	Ser	Ala	Pro	Arg	Ser	Trp	Lys	Gly	Met	Asn	Ser	Gln
		195					200					205			

Val Ala Ser Ser Leu Gln Gln Pro Ser Asn Leu Asp Leu Pro Pro Ala
210 215 220

Pro Glu Leu Asp Trp Met Glu Thr Gly Pro Ser Leu Thr Phe Ile Gly
225 230 235 240

His Gln Asp Ile Pro Gly Val Gly Asn Ile His Ser Gly Ala Thr Pro
245 250 255

Pro Trp Met Ile Gln Asp Glu Glu Tyr Ile Ala Gly Asn Gln Glu Ile
260 265 270

Gly Pro Ser Tyr Glu Glu Phe Leu Lys Glu Lys Glu Lys Gln Lys Leu
275 280 285

Lys Lys Leu Pro Pro Asp Arg Val Gly Ala Asn Phe Asp His Ser Ser
290 295 300

Arg Thr Ser Ala Gly Trp Leu Pro Ser Phe Gly Pro Arg Leu Glu Xaa
305 310 315 320

Trp Thr Pro Leu Ala Val Gln Thr Ser Thr Pro Lys Leu Lys Leu Gln
325 330 335

Gln Xaa Arg Ser Ser His Ile Gln Lys Lys Ala Asn His Ala Leu Tyr
340 345 350

Gln Leu Pro Xaa Gly Xaa Lys Pro Lys Ser Thr Lys Pro Leu Leu Tyr
355 360 365

Leu Pro Pro Lys Phe Phe Ile Ile Val Phe Leu Arg Lys Gln Thr Tyr
370 375 380

Ser Phe Ile Xaa Phe Asn Lys Val Leu Phe Phe Gly Leu Arg Gly Leu
385 390 395 400

Glu Ser Ser Leu Ser Ile Pro Ser Thr Ser Arg Gly Gly Arg Thr His
405 410 415

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(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 4596 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

AGCGGGGGGA CTGTGCCGTG TGGACGTGT AGCTGTTGAA GGTGGACTCT GTTACCATTG	60
AGGATGTTTG GAGGATGAGT ATGTGTGGCA GAGGCACACA TAAACAGGCA GAGACCCCTT	120

GGCCCTGCCT	TTCTCCCCCA	ACCCAAGGCT	GACCTGTGTT	CTCCCAGGTC	TGGGATTCTA	180
AGTGACCTGC	TCTGTGTTTG	GTCTCTCTCA	GGATGAGCAC	AAGCCTGGGA	GATGGCAGTG	240
ATGGAATG	CCTGCCCAGG	TGCCCCTGCC	TCAGCAGTGG	GGCAGCAGAA	GGAAGTCCCC	300
AAGCCAAAG	AGAAGACGCC	GCCACTGGGG	AAGAAACAGA	GCTCCGCTCA	CAAGCTTGAG	360
GCCGTGGAGA	AGAGCCCTGT	GTTCTGCGGA	AAGTGGGAGA	TCCTGAATGA	CGTGATTACC	420
AAGGGCACAG	CCAAGGAAGG	CTCCGAGGCA	GGGCCAGCTG	CCATCTCTAT	CATGCCCCAG	480
GCTGAGTGTG	AGAATAGCCA	AGAGTTCAGC	CCCACCTTTT	CAGAACGCAT	TTTCATCGCT	540
GGGTCCAAC	AGTACAGCCA	GTCCGAGAGT	CTTGATCAGA	TCCCCAACAA	TGTGGCCCAT	600
GCTACAGAGG	GCAAAATGCG	CCGTGTGTGT	TGGAAGGGAA	AGCGTCGCAG	CAAAGCCCGG	660
AAGAAACGGA	AGAAGAAGAG	CTCAAAGTCC	CTGGCTCATG	CAGGAGTGGC	CTTGGCCAAA	720
CCCCTCCCCA	GGACCCCTGA	GCAGGAGAGC	TGCACCATCC	CAGTGCAGGA	GGATGAGTCT	780
CCACTCGGCG	CCCCATATGT	TAGAAACACC	CCGCAGTTCA	CCAAGCCTCT	GAAGGAACCA	840
GGCCTTGGGC	AACTCTGTTT	TAAGCAGCIT	GGCGAGGGCC	TACGGCCGGC	TCTGCCTCGA	900
TCAGAACTCC	ACAAACTGAT	CAGCCCCCTG	CAATGTCTGA	ACCACGTGTG	GAAACTGCAC	960
CACCCCCAGG	ACGGAGGGCC	CCTGCCCTTG	CCCACGCACC	CCTTCCCCCTA	TAGCAGACTG	1020
CCTCATCCCT	TCCCATTCCA	CCCTCTCCAG	CCCTGGAAAC	CTCACCTCTT	GGAGTCTCTC	1080
CTGGGCAAA	TGGCCTGTGT	AGACAGCCAG	AAACCCTTGC	CTGACCCACA	CCTGAGCAAA	1140
CTGGCCTGTG	TAGACAGTCC	AAAGCCCTTG	CCTGGCCAC	ACCTGGAGCC	CAGCTGCCTG	1200
TCTCGTGGTG	CCCATGAGAA	GTTTTCTGTG	GAGGAATACC	TAGTGCATGC	TCTGCAAGGC	1260
AGCGTGAGCT	CAAGCCAGGC	CCACAGCCTG	ACCAGCCTGG	CCAAGACCTG	GGCAGCACGG	1320
GGCTCCAGAT	CCCGGGAGCC	CAGCCCCAAA	ACTGAGGACA	ACGAGGGTGT	CCTGCTCACT	1380
GAGAACTCA	AGCCAGTGGA	TTATGAGTAC	CGAGAAGAAG	TCCACTGGGC	CACGCACCAG	1440
CTCCGCCTGG	GCAGAGGCTC	CTTCGAGAG	GTGCACAGGA	TGGAGGACAA	GCAGACTGGC	1500
TTCCAGTGGC	CTGTCAAAAA	GGTGCGCCCT	GAAGTATTTC	GGGCAGAGGA	GCTGATGGCA	1560
TGTGCAGGAT	TGACCTCACC	CAGAATTGTC	CCTTTGTATG	GAGCTGTGAG	AGAAGGGCCT	1620
TGGGTCAACA	TCTTCATGGA	GCTGCTGGAA	GGTGGCTCCC	TGGGCCAGCT	GGTCAAGGAG	1680
CAGGGCTGTC	TCCCAGAGGA	CCGGGCCCTG	TACTACCTGG	GCCAGGCCCT	GGAGGGTCTG	1740
GAATACCTCC	ACTCACGAAG	GATTCTGCAT	GGGGACGTCA	AAGCTGACAA	CGTGCTCCTG	1800
TCCAGCGATG	GGAGCCACGC	AGCCCTCTGT	GACTTTGGCC	ATGCTGTGTG	TCTTCAACCT	1860
GATGGCCTGG	GAAAGTCCTT	GCTCACAGGG	GACTACATCC	CTGGCACAGA	GACCCACATG	1920

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TGTGCCCTT TGCTGCTGGC TACCACTCTT CCCCAGAGCA GCAGCCCCCG AGCCCCTTCA	3840
GCCCCAGCAC TGCCCCAGAC TCGCTGGCAC TCAGTTCCT CATCTGTAA GGTGAAGGTT	3900
GATGCAGGAT ATGCCTGACA GGAACAGTCT GTGGATGGAC ATGATCAGTG CTAAGGAAAG	3960
CAGCAGAGAG AGACGTCOGG CGCCCCAGCC CCACTATCAG TGTCCAGCGT GCTGGTTCCC	4020
CAGAGCACAG CTCAGCATCA CACTGACACT CACCTGCCC TGCCCCTGGC CAGAGGGTAC	4080
TGCGACGGC ACTTTGCACT CTGATGACCT CAAAGCACTT TCATGGCTGC CCTCTGGCAG	4140
GGCAGGCGAC GGCAGTGACA CTGTAGGAGC ATAGCAAGCC AGGAGATGGG GTGAAGGGAC	4200
ACAGTCTTGA GCTGTCCACA TGCATGTGAC TCCTCAAACC TCTTCCAGAT TTCTCTAAGA	4260
ATAGACCCCC CTTCCTCATT GCCCCAGCTT AGCCTCTTCT CCCAGGGGAG CTACTCAGGA	4320
CTCACGTAGC ATTAATCAG CTGTGAATCG TCAGGGGGTG TCTGCTAGCC TCAACCTCCT	4380
GGGGCAGGGG ACGCCGAGAC TCCGTGGGAG AAGCTCATTC CCACATCTTG CCAAGACAGC	4440
CTTTGTCCAG CTGTCCACAT TGAGTCAGAC TGCTCCCGGG GAGAGAGCCC CGGCCCCCAG	4500
CACATAAAGA ACTGCAGCCT TGGTACTGCA GAGTCTGGGT TGTAGAGAAC TCTTTGTAAG	4560
CAATAAAGTT TGGGGTGATG ACAAATGTTA AAAAAA	4596

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 947 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

Met	Ala	Val	Met	Glu	Met	Ala	Cys	Pro	Gly	Ala	Pro	Gly	Ser	Ala	Val
1				5					10					15	
Gly	Gln	Gln	Lys	Glu	Leu	Pro	Lys	Pro	Lys	Glu	Lys	Thr	Pro	Pro	Leu
			20						25					30	
Gly	Lys	Lys	Gln	Ser	Ser	Val	Tyr	Lys	Leu	Glu	Ala	Val	Glu	Lys	Ser
			35						40					45	
Pro	Val	Phe	Cys	Gly	Lys	Trp	Glu	Ile	Leu	Asn	Asp	Val	Ile	Thr	Lys
			50						55					60	
Gly	Thr	Ala	Lys	Glu	Gly	Ser	Glu	Ala	Gly	Pro	Ala	Ala	Ile	Ser	Ile
			65						70					80	
Ile	Ala	Gln	Ala	Glu	Cys	Glu	Asn	Ser	Gln	Glu	Phe	Ser	Pro	Thr	Phe
			85						90					95	

Ser Glu Arg Ile Phe Ile Ala Gly Ser Lys Gln Tyr Ser Gln Ser Glu
 100 105 110
 Ser Leu Asp Gln Ile Pro Asn Asn Val Ala His Ala Thr Glu Gly Lys
 115 120 125
 Met Ala Arg Val Cys Trp Lys Gly Lys Arg Arg Ser Lys Ala Arg Lys
 130 135 140
 Lys Arg Lys Lys Lys Ser Ser Lys Ser Leu Ala His Ala Gly Val Ala
 145 150 155 160
 Leu Ala Lys Pro Leu Pro Arg Thr Pro Glu Gln Glu Ser Cys Thr Ile
 165 170 175
 Pro Val Gln Glu Asp Glu Ser Pro Leu Gly Ala Pro Tyr Val Arg Asn
 180 185 190
 Thr Pro Gln Phe Thr Lys Pro Leu Lys Glu Pro Gly Leu Gly Gln Leu
 195 200 205
 Cys Phe Lys Gln Leu Gly Glu Gly Leu Arg Pro Ala Leu Pro Arg Ser
 210 215 220
 Glu Leu His Lys Leu Ile Ser Pro Leu Gln Cys Leu Asn His Val Trp
 225 230 235 240
 Lys Leu His His Pro Gln Asp Gly Gly Pro Leu Pro Leu Pro Thr His
 245 250 255
 Pro Phe Pro Tyr Ser Arg Leu Pro His Pro Phe Pro Phe His Pro Leu
 260 265 270
 Gln Pro Trp Lys Pro His Pro Leu Glu Ser Phe Leu Gly Lys Leu Ala
 275 280 285
 Cys Val Asp Ser Gln Lys Pro Leu Pro Asp Pro His Leu Ser Lys Leu
 290 295 300
 Ala Cys Val Asp Ser Pro Lys Pro Leu Pro Gly Pro His Leu Glu Pro
 305 310 315 320
 Ser Cys Leu Ser Arg Gly Ala His Glu Lys Phe Ser Val Glu Glu Tyr
 325 330 335
 Leu Val His Ala Leu Gln Gly Ser Val Ser Ser Ser Gln Ala His Ser
 340 345 350
 Leu Thr Ser Leu Ala Lys Thr Trp Ala Ala Arg Gly Ser Arg Ser Arg
 355 360 365
 Glu Pro Ser Pro Lys Thr Glu Asp Asn Glu Gly Val Leu Leu Thr Glu
 370 375 380
 Lys Leu Lys Pro Val Asp Tyr Glu Tyr Arg Glu Glu Val His Trp Ala
 385 390 395 400
 Thr His Gln Leu Arg Leu Gly Arg Gly Ser Phe Gly Glu Val His Arg
 405 410 415

Met Glu Asp Lys Gln Thr Gly Phe Gln Cys Ala Val Lys Lys Val Arg
420 425 430

Leu Glu Val Phe Arg Ala Glu Glu Leu Met Ala Cys Ala Gly Leu Thr
435 440 445

Ser Pro Arg Ile Val Pro Leu Tyr Gly Ala Val Arg Glu Gly Pro Trp
450 455 460

Val Asn Ile Phe Met Glu Leu Leu Glu Gly Gly Ser Leu Gly Gln Leu
465 470 475 480

Val Lys Glu Gln Gly Cys Leu Pro Glu Asp Arg Ala Leu Tyr Tyr Leu
485 490 495

Gly Gln Ala Leu Glu Gly Leu Glu Tyr Leu His Ser Arg Arg Ile Leu
500 505 510

His Gly Asp Val Lys Ala Asp Asn Val Leu Leu Ser Ser Asp Gly Ser
515 520 525

His Ala Ala Leu Cys Asp Phe Gly His Ala Val Cys Leu Gln Pro Asp
530 535 540

Gly Leu Gly Lys Ser Leu Leu Thr Gly Asp Tyr Ile Pro Gly Thr Glu
545 550 555 560

Thr His Met Ala Pro Glu Val Val Leu Gly Arg Ser Cys Asp Ala Lys
565 570 575

Val Asp Val Trp Ser Ser Cys Cys Met Met Leu His Met Leu Asn Gly
580 585 590

Cys His Pro Trp Thr Gln Phe Phe Arg Gly Pro Leu Cys Leu Lys Ile
595 600 605

Ala Ser Glu Pro Pro Pro Val Arg Glu Ile Pro Pro Ser Cys Ala Pro
610 615 620

Leu Thr Ala Gln Ala Ile Gln Glu Gly Leu Arg Lys Glu Pro Ile His
625 630 635 640

Arg Val Ser Ala Ala Glu Leu Gly Gly Lys Val Asn Arg Ala Leu Gln
645 650 655

Gln Val Gly Gly Leu Lys Ser Pro Trp Arg Gly Glu Tyr Lys Glu Pro
660 665 670

Arg His Pro Pro Pro Asn Gln Ala Asn Tyr His Gln Thr Leu His Ala
675 680 685

Gln Pro Arg Glu Leu Ser Pro Arg Ala Pro Gly Pro Arg Pro Ala Glu
690 695 700

Glu Thr Thr Gly Arg Ala Pro Lys Leu Gln Pro Pro Leu Pro Pro Glu
705 710 715 720

Pro Pro Glu Pro Asn Lys Ser Pro Pro Leu Thr Leu Ser Lys Glu Glu
725 730 735

Ser Gly Met Trp Glu Pro Leu Pro Leu Ser Ser Leu Glu Pro Ala Pro

740										745					750						
Ala	Arg	Asn	Pro	Ser	Ser	Pro	Glu	Arg	Lys	Ala	Thr	Val	Pro	Glu	Gln						
		755					760					765									
Glu	Leu	Gln	Gln	Leu	Glu	Ile	Glu	Leu	Phe	Leu	Asn	Ser	Leu	Ser	Gln						
	770					775					780										
Pro	Phe	Ser	Leu	Glu	Glu	Gln	Glu	Gln	Ile	Leu	Ser	Cys	Leu	Ser	Ile						
	785				790					795					800						
Asp	Ser	Leu	Ser	Leu	Ser	Asp	Asp	Ser	Glu	Lys	Asn	Pro	Ser	Lys	Ala						
			805						810					815							
Ser	Gln	Ser	Ser	Arg	Asp	Thr	Leu	Ser	Ser	Gly	Val	His	Ser	Trp	Ser						
			820					825						830							
Ser	Gln	Ala	Glu	Ala	Arg	Ser	Ser	Ser	Trp	Asn	Met	Val	Leu	Ala	Arg						
		835					840					845									
Gly	Arg	Pro	Thr	Asp	Thr	Pro	Ser	Tyr	Phe	Asn	Gly	Val	Lys	Val	Gln						
	850					855					860										
Ile	Gln	Ser	Leu	Asn	Gly	Glu	His	Leu	His	Ile	Arg	Glu	Phe	His	Arg						
	865				870					875					880						
Val	Lys	Val	Gly	Asp	Ile	Ala	Thr	Gly	Ile	Ser	Ser	Gln	Ile	Pro	Ala						
				885					890					895							
Ala	Ala	Phe	Ser	Leu	Val	Thr	Lys	Asp	Gly	Gln	Pro	Val	Arg	Tyr	Asp						
			900					905					910								
Met	Glu	Val	Pro	Asp	Ser	Gly	Ile	Asp	Leu	Gln	Cys	Thr	Leu	Ala	Pro						
		915				920						925									
Asp	Gly	Ser	Phe	Ala	Trp	Ser	Trp	Arg	Val	Lys	His	Gly	Gln	Leu	Glu						
	930					935					940										
Asn	Arg	Pro																			
	945																				

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

- (A) DESCRIPTION: /desc = "oligonucleotide PCR primer"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

CAGGATCCTC ATGGCTGCAG CTAGCGTGAC

30

(2) INFORMATION FOR SEQ ID NO: 9:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 32 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(A) DESCRIPTION: /desc = "oligonucleotide PCR primer"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

GGTCGACTTA GAGCCCTGTC AGGTCCACAA TG

32

(2) INFORMATION FOR SEQ ID NO: 10:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(A) DESCRIPTION: /desc = "oligonucleotide probe"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

GATGCCATTG GGGATTTCCT CTTT

24

(2) INFORMATION FOR SEQ ID NO: 11:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(A) DESCRIPTION: /desc = "oligonucleotide probe"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

CAGTAAAGAG GAAATCCCCA ATGG

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Combined Declaration for Patent Application and Power of Attorney

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name; and that I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled

MODULATORS OF TNF RECEPTOR ASSOCIATED FACTOR (TRAF), THEIR PREPARATION AND USE
the specification of which (check one)

[] is attached hereto;

[] was filed in the United States under 35 U.S.C. §111 on _____, as
USSN _____; or

[X] was/will be filed in the U.S. under 35 U.S.C. §371 by entry into the U.S. national stage of
an international (PCT) application, PCT/IL97/00117; filed 01 April 1997,
entry requested on 02 October 1998; national stage application received
USSN _____; §371/§102(e) date _____ (*if known),

and was amended on 02 October 1998 (if applicable).

(include dates of amendments under PCT Art. 19 and 34 if PCT)

I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above; and I acknowledge the duty to disclose to the Patent and Trademark Office (PTO) all information known by me to be material to patentability as defined in 37 C.F.R. § 1.56.

I hereby claim foreign priority benefits under 35 U.S.C. §§ 119, 365 of any prior foreign application(s) for patent or inventor's certificate, or prior PCT application(s) designating a country other than the U.S., listed below with the "Yes" box checked and have also identified below any such application having a filing date before that of the application on which priority is claimed:

117800 (Number)	Israel (Country)	02 April 1996 (Day Month Year Filed)	[X] YES	[] NO
119133 (Number)	Israel (Country)	26 August 1996 (Day Month Year Filed)	[X] YES	[] NO

I hereby claim the benefit under 35 U.S.C. § 120 of any prior U.S. non-provisional Application(s) or prior PCT Application(s) designating the U.S. listed below, or under § 119(e) of any prior U.S. provisional applications listed below, and, insofar as the subject matter of each of the claims of this application is not disclosed in such U.S. or PCT application in the manner provided by the first paragraph of 35 U.S.C. § 112, I acknowledge the duty to disclose to the PTO all information as defined in 37 C.F.R. § 1.56(a) which occurred between the filing date of the prior application and the national filing date of this application:

(Application Serial No.)	(Day Month Year Filed)	(Status: patented, pending, abandoned)
(Application Serial No.)	(Day Month Year Filed)	(Status: patented, pending, abandoned)
(Application Serial No.)	(Day Month Year Filed)	(Status: patented, pending, abandoned)

I hereby appoint the following attorneys, with full power of substitution, association, and revocation, to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith.

SHERIDAN NEIMARK, REG. NO. 20,520 - ROGER L. BROWDY, REG. NO. 25,618 - ANNE M. KORNBAU, REG. NO. 25,884
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The undersigned hereby authorizes the U.S. Attorneys or Agents named herein to accept and follow instructions from INTER-LAB LTD. as to any action to be taken in the U.S. Patent and Trademark Office regarding this application without direct communication between the U.S. Attorney or Agent and the undersigned. In the event of a change of the persons from whom instructions may be taken, the U.S. Attorneys or Agents named herein will be so notified by the undersigned.

Title: MODULATORS OF TNF RECEPTOR ASSOCIATED FACTOR (TRAF). THEIR...

U.S. Application filed _____, Serial No. _____

PCT Application filed 01 April 1997 _____, Serial No. PCT/IL97/00117

I hereby further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. §1001 and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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POST OFFICE ADDRESS		
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RESIDENCE	CITIZENSHIP	
POST OFFICE ADDRESS		